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LONG-TERM BIOEFFECTS OF 435-MHz RADIOFREQUENCY RADIATION ON SELECTED BLOOD-BORNE ENDPOINTS IN CANNULATED RATS

Volume 2. Plasma ACTH and Plasma Corticosterone

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NOTICES

This final report was submitted by Georgia Tech Research Institute, Georgia Institute of Technology, Atlanta, Georgia, under contract F33615-83-K-0600, job order 7757-01-78, with the USAF School of Aerospace Medicine, Human Systems Division, AFSC, Brooks Air Force Base, Texas. James H. Merritt (USAFSAM/RZP) was the Laboratory Project Scientist-in-Charge.

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The animals involved in this study were procured, maintained, and used in accordance with the Animal Welfare Act and the "Guide for the Care and Use of Laboratory Animals" prepared by the Institute of Laboratory Animal Resources-National Research Council.

The Office of Public Affairs has reviewed this report, and it is releasable to the National Technical Information Service, where it will be available to the general public, including foreign nationals.

This report has been reviewed and is approved for publication.

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Two hundred adult male white rats (Sprague-Dawley, CAMM Labs) with chronically implanted aortic cannulas were randomly divided into two groups. Animals in the first group were exposed to low-level (1.0 mW/cm^2) pulsed-wave 435-MHz radiofrequency radiation (RFR) for approximately 22 h daily, 7 days each week, for 6 months. Animals in the second group were maintained under identical conditions, but were not radiated. The cannulas were used to draw microsamples (0.3 mL) of aortic blood from the unrestrained, unanesthetized rats on a cyclic schedule. Plasma adrenocorticotropic hormone (ACTH) and plasma corticosterone concentrations were determined by radioimmunoassays. Statistical analysis of the results did not indicate increased plasma ACTH and plasma corticosterone concentrations in exposed animals when compared to sham-exposed animals. Exposure to this low-level radiofrequency environment did not induce stresses that were manifested as an alteration in plasma hormones.

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I. INTRODUCTION

During the past 50 years, the United States has witnessed a period of explosive growth in the radar and communications fields. This growth has increased the demand for available bandwidth, and this has pushed radar and communications frequencies into higher and higher ranges. Higher frequency ranges have permitted faster data transmission rates and reduced intersystem electromagnetic interference. However, these advances have come at the expense of altering the planet's radiofrequency radiation (RFR) environment. Until the advent of advanced radar and communications, cosmic rays and background radiation were the primary sources of the Earth's electromagnetic environment. Radar and communications transmissions have since increased the electromagnetic background or ambient radiation at the planet's surface by several orders of magnitude. At this time, the biological effects of exposure to this omnipresent electromagnetic environment are not well understood, despite studies conducted over the past several decades.

This report presents results of plasma adrenocorticotropic hormone (ACTH) and plasma corticosterone assays of blood samples drawn from a large population of male Sprague-Dawley rats exposed to a 1.0 mW/cm^2, 435-MHz pulsed-wave (1.0-μs pulse width, 1-kHz pulse rate) RFR environment for a 6-month duration. The exposure group consisted of 100 cannulated rats housed in Plexiglas cages arrayed on the tiers of a stacked, parallel-plate circular waveguide. Engineering aspects of this waveguide and the exposure environment it generated have been previously reported [1]. The sham-exposure group consisted of 100 cannulated rats housed in an identical, but unenergized, collocated facility. Results reporting plasma prolactin concentrations in these same animals are in Volume 3 of this series [2].

The pituitary gland releases a number of hormones that regulate physiological functions in the body. One of these hormones, adrenocorticotrophic hormone (ACTH), stimulates the adrenal cortex to release corticosterone and other cortical hormones, such as cortisol. In rats and mice, only corticosterone is secreted. The primary physiologic role of ACTH is to

stimulate the secretion and synthesis of corticosterone (and of cortisol in man and in many animal species except the rat) by the adrenal cortex. ACTH also acts as a trophic substance maintaining the size and blood flow to the adrenal cortex and exhibits adrenal physiologic effects on cyclic-adenosine monophosphate (c-AMP) mediated systems. Both ACTH and corticosterone are readily affected by stress. In all species observed so far and in all experimental situations, a stressful condition leads to an increased secretion of ACTH [3] and of corticosterone [4,5]. The increase in stress hormones corresponds to the stimulus, being a graded function of stimulus intensity and duration [6]. Depending on intensity (and duration) of stress, plasma concentration of the hormones is increased several-fold. This fact permits the increase of plasma ACTH and corticosterone concentration to be used to measure stress intensity. In rats, levels of plasma corticosterone vary from 6-8 μg/100 mL in unstressed animals to 80 μg/100 mL in stressed animals, a tenfold increase. This increase indicates how sensitive the hypothalamic-pituitary-adrenal axis is to physiological and psychological insults [7].

Anesthesia, exercise [8], immobilization [9], withdrawal of large volumes of blood [10], exposure to a new, unfamiliar cage or room [11,12], noise, hypoxia [13,14], a decreased Pa_{O_2}, handling of the animals [15], cold exposure [16], and many other environmental factors lead to increases in plasma concentration of ACTH and corticosterone and, therefore, had to be avoided in this study. Even smoking in the animal housing room increases rat plasma ACTH and plasma corticosterone levels [17]. Clearly, both neurogenic (emotional) and systemic (somatic) stimuli are effective in evoking increased ACTH and corticosterone secretion in animals and in man.

Circadian rhythm is a known factor affecting plasma hormone concentrations in rats. Both plasma ACTH and plasma corticosterone follow circadian rhythm, rising each day during the evening hours and decreasing to the lowest level between 9 AM and 1 PM [18,19].

Some researchers have suggested that individual housing might lead to increases in plasma corticosterone in rats (called "isolation-stress syndrome," [20,21,22]). However, most investigators found the difference between single- or group-housed rats to be very small or nonexistent. Results of experiments during this study also indicated that values of resting plasma ACTH and plasma corticosterone were similar in both single- and in group-housed rats.

The ACTH and corticosterone increase in response to stress can be quantified. The degree of plasma ACTH or plasma corticosterone increase is related to the type of stress to which the animal is exposed [11].

II. MATERIALS AND METHODS

For this study, the concentrations of both plasma ACTH and plasma corticosterone were chosen as sensitive indicators of possible environmental stresses induced by RFR. To detect and quantitatively evaluate possible increases in plasma ACTH and plasma corticosterone levels induced by RFR, blood was sampled and assayed from 86 exposed and 65 sham-exposed animals (in the case of ACTH); 87 exposed and 65 sham-exposed animals (in the case of corticosterone). The animals assayed for ACTH concentration correspond to the animals assayed for corticosterone concentration. Analysis of the data obtained from the blood sample assays determined whether there were any RFR-induced changes in plasma ACTH and plasma corticosterone concentrations.

Animals. The rat represents a comparatively inexpensive and homogeneous population. For this reason, it is often desirable to use this species as the animal model in physiologic studies.

We used male Sprague-Dawley rats in this study. All experimental animals were obtained from the same building and room at CAMM Research Labs, Wayne, New Jersey. The animals, weighing approximately 60 g, were delivered to Emory University where they were caged singly and given water and food (Purina Rat Chow) ad libitum. Temperature in the animal rooms was maintained at 24 $\pm$ 1 ^{o}C and the photoperiod was 12 hours/12 hours, with the lighted phase occurring between 8 AM and 8 PM.

Experimental Facility. The Georgia Tech Research Institute's Radiofrequency Radiation Facility [23] consisted of 8 collocated rooms on the basement floor of the Baker Building on the main campus. These eight rooms provided a closed, complete facility for long-term bioeffects studies involving rodents.

Two identical, collocated rooms in the Facility housed the 100 exposure and 100 sham-exposure animals. Each room contained a stack of circular, parallel-plate waveguides fed by a slotted-cylinder antenna system for radiating the animals. The stacks of parallel waveguides consisted of five 3.6-m (12 ft) dia. plates that made up 4 sets of circular waveguides. Twenty-five individually housed rats were positioned around the circumference of each waveguide set. The walls of both rooms were lined with anechoic absorbing material and shielded with aluminum foil to prevent excessive microwave leakage radiation.

The circular, parallel-plate waveguide assembly provided a 1.0 mW/cm^2 exposure field around the circumference of the plates. The 45.7-cm (18 in.) plate separation distance permitted propagation of a TE_{10} mode wave with horizontal polarization. The power density displayed a cosine-squared dependency between the plates, with the maximum power density occurring midway between each set of plates. This arrangement positioned the electric field vector parallel to the rat's longitudinal axis, thereby maximizing the coupling between the electric field and the rat.

A slotted-cylinder antenna with the proper diameter, thickness, slot length, and slot width dimensions fed the stack of circular waveguides in a manner that provided an essentially constant electric field intensity in the azimuth plane.

Cages. The cages were constructed of Plexiglas to facilitate visual observation of the rats. Each cage was 22.9-cm (9 in.) long by 12.7-cm (5 in.) wide by 17.8-cm (7 in.) tall. These dimensions complied with dimensions recommended by the National Institutes of Health for long-term housing of rats [24]. The food hopper and water bottle were placed on the distal side of the cage to minimize their interaction with the exposure field. The glass floor rods in the cage were oriented perpendicular to the cage's long axis to induce the rats to preferentially align themselves parallel to the electric field vector. The sipper tubes of the water bottles were made of glass to be nonperturbing in the field. Evaluations of the cages conducted in the circular, parallel-plate waveguide assembly showed field scattering from the Plexiglas and water to be below the range of detection.

The RFR Facility contained a data acquisition system for storing and processing experimental data, an electronic balance for weighing the rats during the study, and rooms for transmitter operation, blood sampling, cage washing, and materials storage.

Noise increases the concentration of both plasma ACTH and plasma corticosterone in rats (Fig. 1). To avoid the possible effects of noise during this study, the entire RFR Facility was kept locked to avoid unauthorized entry. Only the animal caretaker and the technician who sampled blood from the animals were permitted uncontrolled entry to the Facility.

Cannulation. To detect and quantitatively evaluate increases in plasma ACTH and plasma corticosterone, the resting levels of these hormones first had to be determined. It became immediately clear that, to obtain the real resting

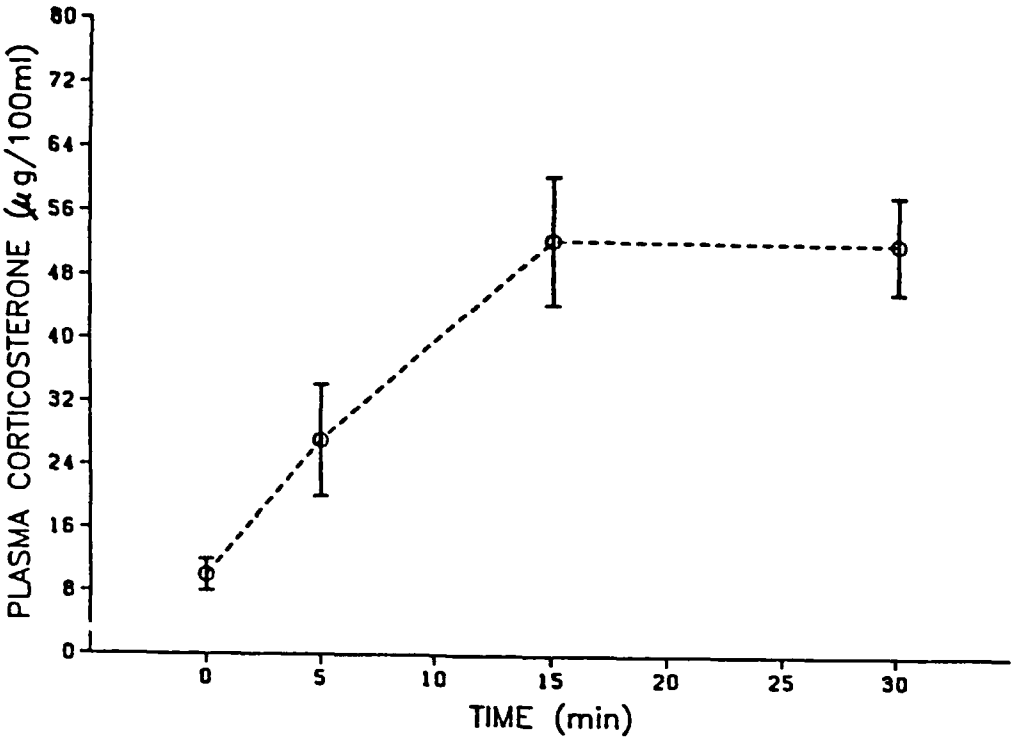


Figure 1. Effect of loud on-off noise on plasma corticosterone in cannulated rats (N = 10).

values of these two hormones in undisturbed animals, many routine techniques for handling the animals and for sampling the blood could not be used. For example, guillotine blood sampling techniques commonly employed in many endocrinological studies were not suitable for this study. To use each animal as its own control, arterial blood was sampled by means of chronically implanted aortic cannulas [25,26]. This simple, inexpensive technique permitted remote stress-free blood sampling in conscious, unrestrained, and resting rats. Arterial blood drawn from the chronically implanted cannulas was assayed for plasma ACTH and plasma corticosterone.

The idea of sampling venous blood from the animals was abandoned. In venous blood vessels, the flow regime is laminar with blood flowing in discrete layers. The layers of blood in the middle of the vessels travel much faster than those close to the vessel walls. The most important consideration, however, was that blood layers do not mix in venous blood vessels. Thus, a sample of venous blood, withdrawn with a needle or a cannula, might represent the blood returning from one part of the body or the other, from a single organ or muscle, or from any one of the endocrine glands. For this reason, we decided to sample arterial blood, which is always fully mixed. The mixing occurs in the left ventricle of the heart and in early parts of the aorta. Only physiologically minute amounts of arterial blood (up to 0.3 mL) were withdrawn from resting rats approximately once every 2 weeks.

PE-10 arterial cannulas were used in this study. Larger PE-50 cannulas were unsuitable because they could develop large blood clots if not drained frequently. Large cannulas require multiple flushing to remain patent, but flushing might induce multiple strokes in the animals. Use of 1000 units/mL heparin in the cannula tip allowed spontaneous patency of PE-50 cannulas to persist only up to 2 days. Chronic cannulation of the aorta with a PE-10 cannula was preferable to cannulation of other arterial blood vessels. The need for properly mixed blood precluded cannulation of either the jugular vein or the inferior vena cava. Cannulation of the abdominal aorta provided long-term functional cannulas [27], but the cannulation procedure was lengthy (20-30 min) and required temporary dislocation of the intestinal system. The abdominal aortic cannula had a much larger dead space than the aortic cannula. Cannulation of the aorta through the left carotid artery, on the other hand, required an incision of 1-1.5 cm that neither penetrated body walls nor entered the abdominal cavity. Further, this cannulation could be completed in about 8 min.

The carotid artery of the animal was cannulated 8 to 10 days before the animals entered the study. The surgery was done using ketamine-xylazine anesthesia (1:1 mixture; ketamine 100 mg/mL, xylazine 20 mg/mL, intramuscular 0.1 mL/100 g of body weight). The catheter was filled with slightly heparinized saline* and the distal end was sealed with a nylon plug. Stress hormone levels returned to the basal values approximately 3 days after implantation of the chronic arterial cannulas. The first blood sampling occurred 10 days after aortic cannulation.

Blood Sampling. Restraint and handling increase stress hormone levels in rats (Fig. 2). This increase will persist 20 to 30 min after the initial stimulus, and is apparent even if the stimulus is removed immediately [7]. However, the animals had to be handled upon removal from their exposure cage and placement in the "sampling box" in preparation for blood withdrawal. To avoid the undesired effects of handling and stress on hormone levels, blood from the aortic cannula was sampled 30 min after the animal was placed in the sampling box. This procedure permitted the altered plasma ACTH and plasma corticosterone levels sufficient time to return to their basal (resting) values. Each animal

*0.5 cm^3 heparin sodium (from beef lung), 1000 units/mL per 30 cm^3 saline.

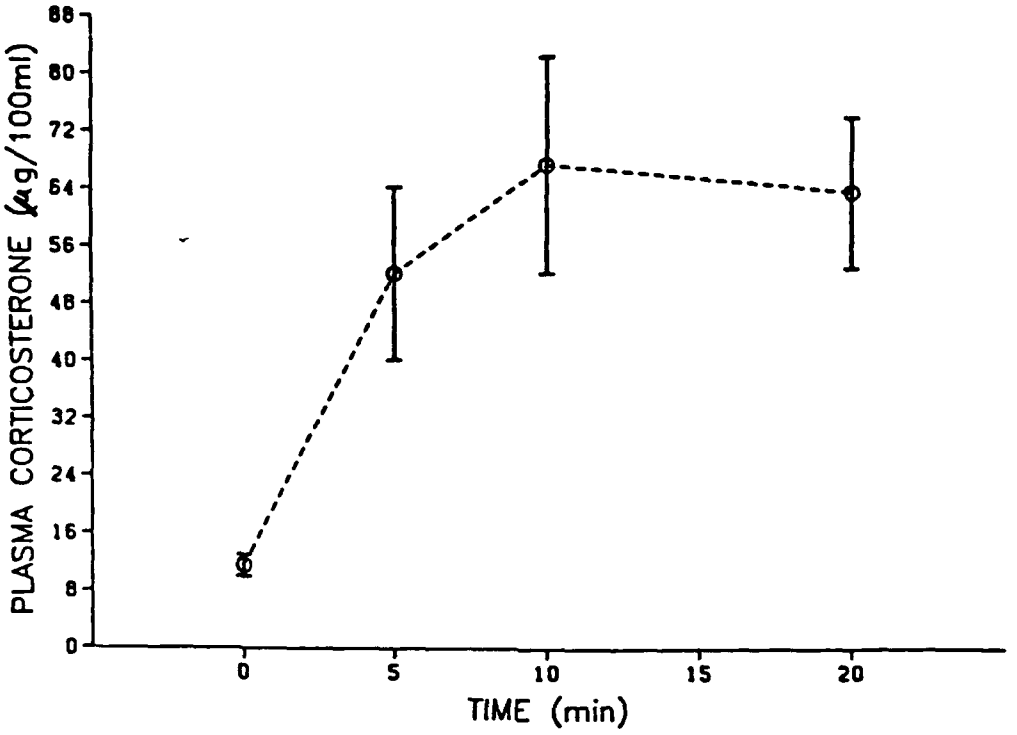


Figure 2. Light restraint (placement of rats in narrow Plexiglas cages) increases plasma corticosterone level of cannulated rats (N = 10).

was preconditioned for the sampling box through a regime of several 30-min-long experiments conducted during a 1-week period before entering the study.

After acclimating for 30 min in the sampling box, the rat's cannula was positioned through the slot in the top of the box (Fig. 3). The heparinized saline was then removed from the cannula, and a 0.3-mL blood sample was taken from the resting rat. The withdrawal of larger amounts of blood from the cannulated rats led to increased plasma corticosterone levels (Fig. 4). Using a sterile 1-cm^3 tuberculin syringe fitted with a 30-ga needle, the blood sample was taken from the cannula. The syringe and the needle were rinsed with ethylenediaminetetracetate (EDTA) before sampling. The blood sample was placed in an EDTA-treated 0.3-mL capillary blood collection container (Walter Sarstedt Co., Princeton, New Jersey), shaken, and then placed on ice. The blood sampling procedure required about 2 min for each rat.

Anesthesia was not used during blood sampling because anesthetic agents affect plasma stress hormone concentrations. In this study, an increase in plasma corticosterone was observed after barbiturate anesthesia (Fig. 5).

Plasma ACTH and corticosterone both follow circadian rhythm in the rat (Fig. 6). To avoid any possible effect of the circadian rhythm, blood sampling occurred between 9 AM and 1 PM only, the period when both stress hormones were at their lowest (true resting) level.

Figure 3. Sampling blood (0.3 mL) from a chronically cannulated rat (the sampling box is transparent to show the animal).

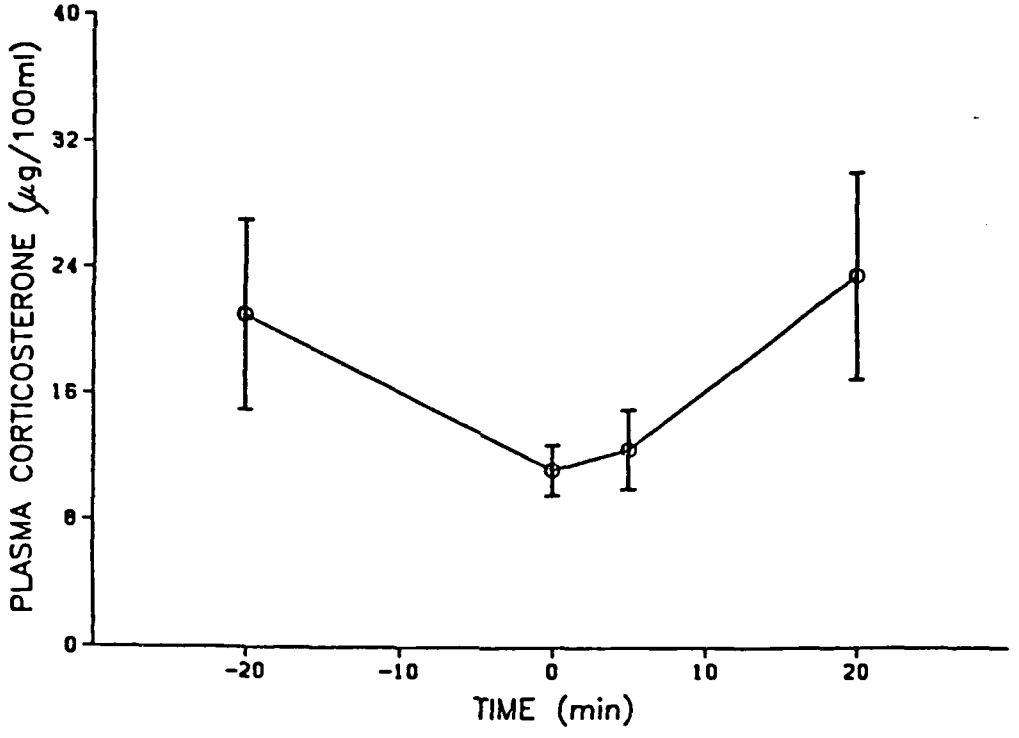


Figure 4. At -20 min 10 cannulated rats were removed from their home cages and placed in sampling boxes. At this moment a first 0.2 mL sample of blood was withdrawn. At 0 time (20 min later) another 0.2 mL sample of blood was withdrawn for plasma corticosterone determination and the animals were bled 1 mL (blood withdrawn from cannula).

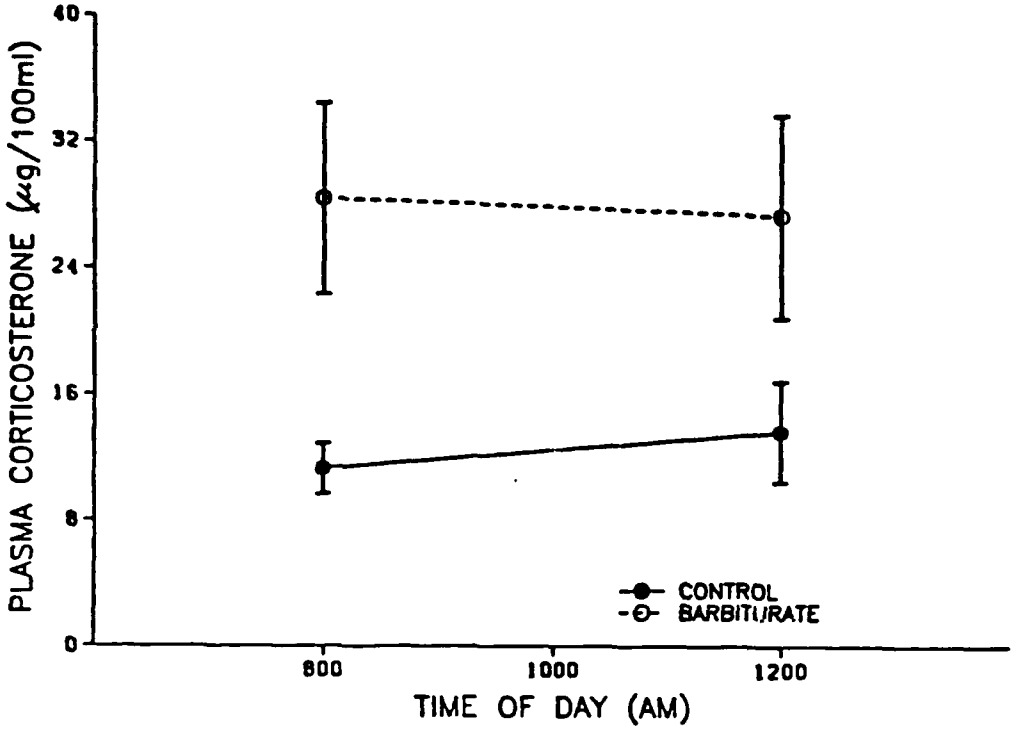


Figure 5. Plasma corticosterone in resting cannulated rats and in the same animals 10 min after administration of light Nembutal anesthesia (2 groups, 10 animals each).

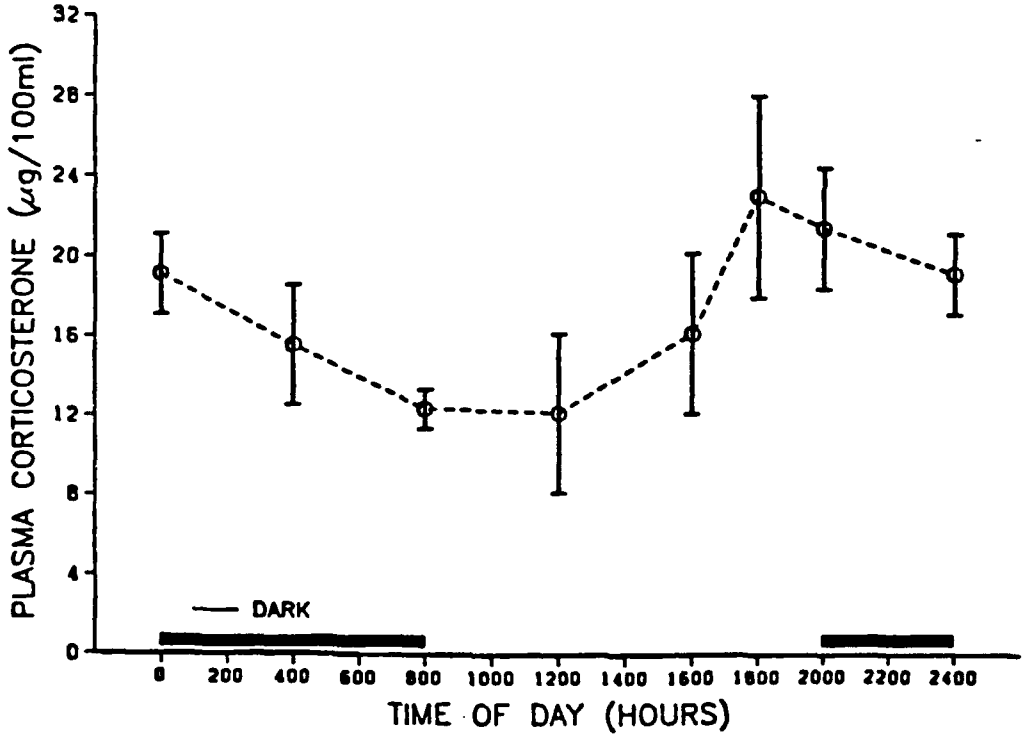


Figure 6. Circadian rhythm of plasma corticosterone in cannulated rats (N=10).

Blood Sampling Schedule. Figure 7 shows the sampling schedule designed for the experiment. Note that the 200 rats were introduced into the study in 4 groups of 50 animals each. The groups entered in a staggered manner to facilitate the process of logging in and establishing the new animals. Each group contained 25 exposure and 25 sham-exposure animals. Of the 25 exposure (or sham-exposure) animals, 20 were sampled for plasma stress hormones, while the remaining 5 were used for hematology studies.

The sampling duration was 36 weeks long, including a 6-week preexposure adaptation period, a 24-week exposure period, and a 6-week postexposure period. Allowing for group staggering, the experiment duration was 42 weeks long (since introduction of the 4 groups was staggered in 2-week intervals). Plasma ACTH and plasma corticosterone were sampled for all time periods marked (A) in Figure 7. Therefore, each animal should have been sampled for both plasma ACTH and plasma corticosterone at weeks -6, -3, 0, 3, 6, ..., 27. This schedule was rather rigorous, and therefore could tolerate slight fluctuations in protocol without ill effects.

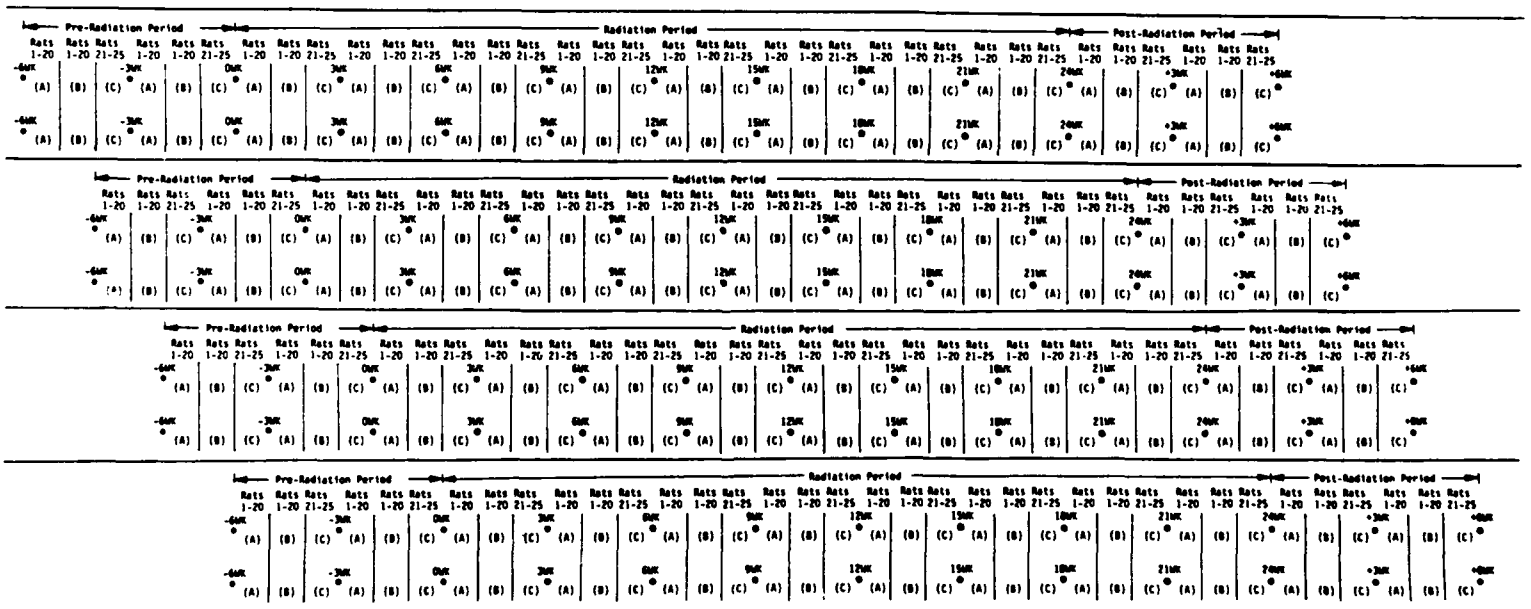


Figure 7. Sampling and exposure timetable.

ACTH and Corticosterone Determinations. Radioimmunoassays are rapid, sensitive, specific, reliable, and require a minimum quantity of blood. The last feature was very important, especially since repetitive sampling was required and small laboratory animals were used.

The first radioimmunoassay was developed and introduced in 1968 by Berson and Yalow [28]. The technique was based on the principle of isotopic dilution in the presence of specific antibodies. Although many variants exist, classical radioimmunoassays of hormones depend upon the competition between radiolabeled and unlabeled analyte (antigen or antigenic substance) for a limited number of binding sites. Provided the binding affinities for the labeled and unlabeled antigens are identical, the quantities of each antigen bound are directly proportional to their relative concentrations. The total concentration of labeled antigen is the same in each incubation mixture of reagents. These reagents are readily available from commercial sources. Reagents for this study were obtained from Immunonuclear Corporation, Stillwater, Maine. With this assay, normal ACTH concentration could be measured in unextracted plasma. The lower limit of sensitivity was determined by the extent to which incubation damage during the assay incubation period could be controlled, since this affected the amount of plasma that the incubation mixture could tolerate.

Plasma ACTH Assay. Following instructions provided by Immunonuclear Corporation, the plasma ACTH assay procedure was as follows:

1. Set up and label 12 x 75 mm disposable glass tubes in duplicate for each standard, including the zero, each control, and each sample.
2. Place the pack of tubes on crushed ice.
3. Add 100 µL of each standard including zero, control or experimental sample to the appropriate tubes.
4. Add 200 µL of rabbit anti-ACTH serum in all tubes except total count tubes.
5. Add 200 µL of ^{125}I-ACTH to all tubes.
6. Mix and incubate for 24 h at 2-8 °C.
7. Add 500 µL goat anti-rabbit precipitating complex to each tube.
8. Mix and incubate for 15-25 min at 20-25 °C.
9. Centrifuge for 20 min at 760 x g at 20-25 °C.
10. Immediately decant the supernate from all tubes except the total count tubes by inverting them for a maximum of 2 min.

11. In a gamma scintillation counter, count the precipitate of each tube and the total count tubes for a sufficient time to achieve statistical accuracy.

12. Run samples with 0, 28, 63, 120, 240, and 600 pg/mL standards and three commercial controls at the beginning and at the end of the assay. In addition, include three pooled rat plasma samples. Standard curves are plotted and values determined by a computer program.

Plasma Corticosterone Assay. The assay for corticosterone was a direct radioimmunoassay for corticosterone which required 25 µL of rat plasma. The method was based on a procedure for cortisol determination in man described by Donohue and Sgoutas [29]. After heat inactivation of corticosterone binding proteins in rat plasma, plasma corticosterone was assayed with specific antisera raised against corticosterone-21-hemisuccinate. While this antibody had a significant cross reactivity with cortisol, prior separation of corticosterone was not necessary since the rat does not secrete cortisol.

The procedural steps included:

A. Preparation of reagents

1. An assay buffer which was 0.05 M Tris, 0.1 M NaCl, and 0.1% BSA, pH 8.0.

2. A boiling buffer which was 0.05 M Tris and 0.1 M NaCl, pH 8.0.

3. (^{3}H) corticosterone (0.25 m Ci, spec. act. 80-100 Ci/mmol; New England Nuclear Corp.) which was diluted to 25 mL with absolute ethanol. For the working solution, 0.1 mL was diluted to 10 mL with assay buffer.

4. An antiserum which was purchased from Miles Laboratories. Each vial was reconstituted with 5 mL assay buffer. The volume of the reconstituted antiserum was diluted with an equal volume of assay buffer for the working solution.

5. Dextran-coated charcoal.

6. Preparation of corticosterone standard by dissolving 10 mg of corticosterone in 500 mL ethanol.

B. Procedure

1. Standards. To a series of tubes labeled A through G, in duplicate, add 0.1 mL of corticosterone standard to tube A, evaporate ethanol under a stream of nitrogen or air, then add 1.25 mL of assay buffer. Vortex well. To the tubes labeled B through G, add 0.5 mL of assay buffer. Add 0.5 mL of the contents of A to tube B. Vortex and transfer 0.5 mL from B to the next tube and so on until the following

serial dilutions of corticosterone are prepared: 160, 80, 40, 20, 10, 5 and 2.5 µg/100 mL.

2. Prepare specimens and controls for assays as follows: Add 0.025 mL of sample and 1.0 mL of boiling buffer in duplicate to appropriately labeled tubes.

3. Cap the standard and sample tubes loosely and place them in a vigorously boiling water bath for 30 min.

4. Label a complete set of tubes including tubes for standards, samples, total counts, and zero point or maximum binding, and counts not absorbed by the charcoal in the absence of antibody or background.

5. Add 0.05 mL of each standard, sample, or control to the appropriate tubes. Add 0.1 mL of radioactive corticosterone working solution to all tubes. Add 0.1 mL of the antiserum working solution into all assay tubes except background and total count tubes.

6. Vortex tubes and place them in a 37 ^{o}C water bath for 60 min.

7. Place tubes in an ice bath for 15 min.

8. Allow Dextran-coated charcoal to stir for 2-3 min before beginning addition. With tubes still in the ice bath, add 0.5 mL of Dextran-coated charcoal to all tubes except total count tubes. Vortex tubes and incubate in ice bath for 10 min after the last addition.

9. Centrifuge all tubes at 3000 rpm at 4 ^{o}C for 10 min.

10. Pour supernate into scintillation vials; add 3.5 mL of scintillation fluid.

11. Count each vial for 10 min in a liquid scintillation counter. The samples are run with three pooled (beginning, middle, and end of run) rat sera to assure within-run and between-run control. Standard curves are plotted and values determined by a computer program with a logit-log plot.

Different volumes of diluted plasma (5, 10, 25, and 50 µL) were assayed and showed good linearity. The intraassay coefficient of variation of a pool containing 10.35 µg/dL was 5.9% (n = 9) and of a pool containing 31.9 µg/dL was 6.6% (n = 9). The interassay variability of the latter pool was 12.0% (n = 8). Accuracy was examined by adding corticosterone, 1.25, 2.50, 5, 10, 20, and 40 µg/dL, to charcoal-treated plasma and assaying the plasma. The regression line was $y = 1.009x + 0.49$, indicating no systematic error.

Resting Concentrations of Plasma ACTH and Plasma Corticosterone. Several groups of preliminary experiments were performed to determine the real resting

value of plasma ACTH and plasma corticosterone in the cannulated rats (Figs. 1, 2, 4, 5, and 6).

There are reports that plasma resting levels of ACTH and corticosterone are smaller in decapitated than in the cannulated rats. However, most investigators have found that the values of plasma ACTH or corticosterone obtained from decapitated rats are similar to the values obtained from cannulated rats. This finding was confirmed during preliminary experiments under this study. Other reports indicate that individually housed, cannulated rats have slightly elevated morning levels of plasma ACTH and corticosterone. Evening or noise-stimulated hormone levels in these rats were similar to those obtained from decapitated rats. In this study, blood sampled between 10 AM and noon from either cannulated animals or from decapitated animals had similar values of plasma corticosterone.

III. RESULTS AND ANALYSIS

Plasma ACTH. Appendix A contains the data collected during the preradiation, radiation, and postradiation periods for both the exposure and sham-exposure groups. The high variance displayed by the data for the entire sampling period indicated various levels of physiological activity at the time of blood sampling. Since the boxes had opaque walls, the activity of each animal prior to sampling was not recorded. However, each animal had sufficient time (30 min or more) to return to basal hormone level after the stimulation of being placed in the sampling box.

Figures 8 and 9 present the raw ACTH concentration data in scatter diagram form (the dotted lines pass through the mean response at each week). Despite efforts to condition the animals to the sampling box environment prior to drawing blood samples, the basal resting value of plasma ACTH decreased during weeks -3, -2, and 0. Beyond this point, the data displayed a generally linear response, factoring out spikes at weeks 7 and 10 (sham-exposure group) and at weeks 8, 12, and 17 (exposure group). The spike at week 10 (sham-exposure group) did not receive much weight since its value derived from a single, "influential," observation. Unusually high personnel activity (with attendant noise) may have been the cause of the remaining spikes.

Plasma ACTH concentrations in the exposure animals did not appear to be greater than plasma ACTH concentrations in the sham-exposure animals (Fig. 10). This was preliminary evidence indicating that 435-MHz RFR did not increase the resting level of plasma ACTH. To attach a numerical probability to this conclusion, a statistical analysis was performed on the data.

Using multiple linear regression procedures, a model was built to describe plasma ACTH concentration as a function of incident RFR and time. Terms of the polynomial model tested for the presence of RFR and time effects. Various statistical diagnostic procedures, including lack-of-fit tests and residual analysis, were then applied to the developed model to check its validity. Appendix B contains detailed descriptions of the methodology, procedures, and results of both the ACTH and corticosterone data analyses.

The statistical analysis indicated that 435-MHz RFR did not increase plasma concentrations of ACTH in the exposure animals when compared to the sham-exposure animals. In fact, the plasma concentration of ACTH in the exposure animals remained lower through most of the experiment when compared to the sham-

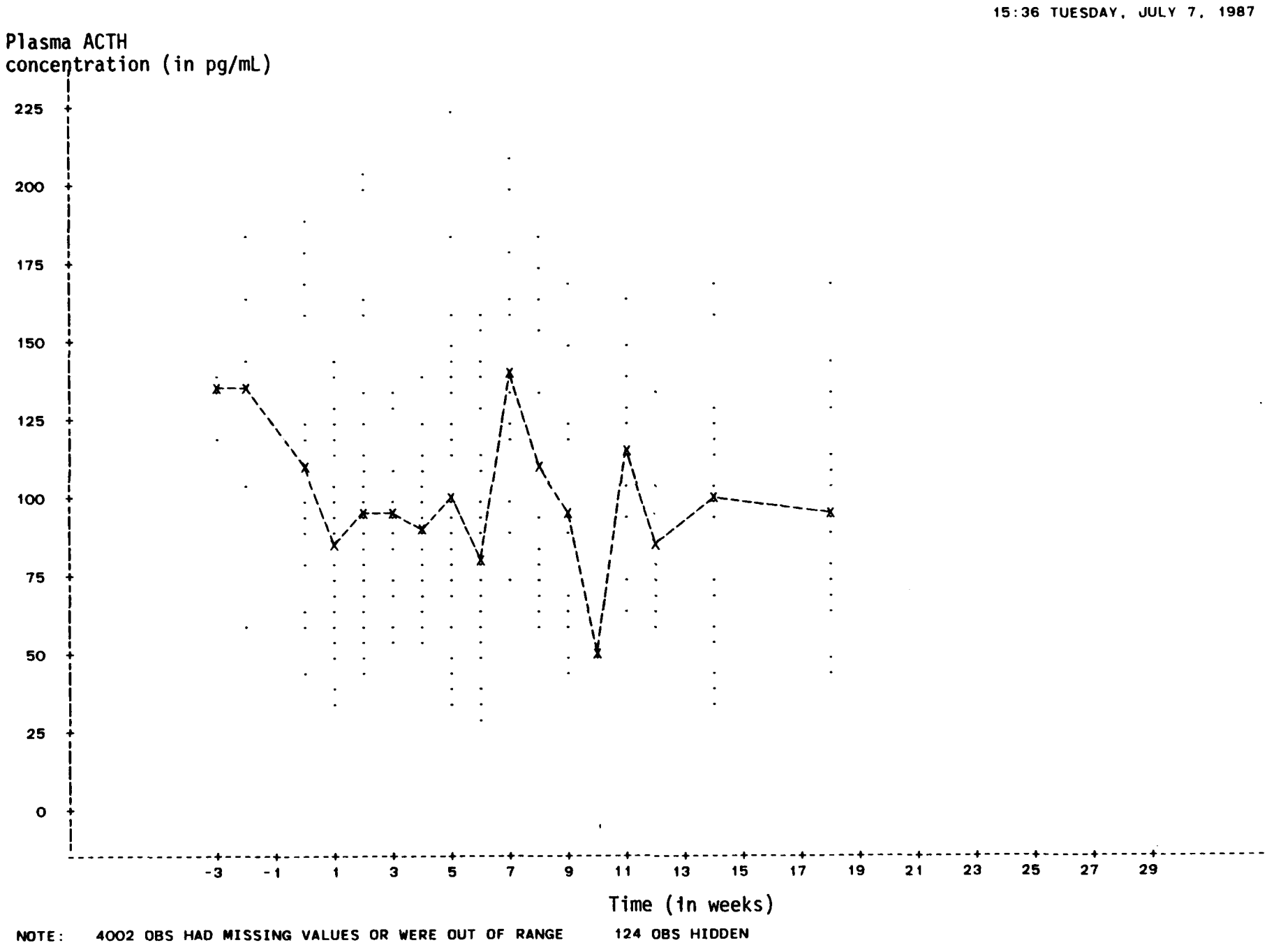


Figure 8. ACTH concentration data scatter diagram (sham-exposure group).

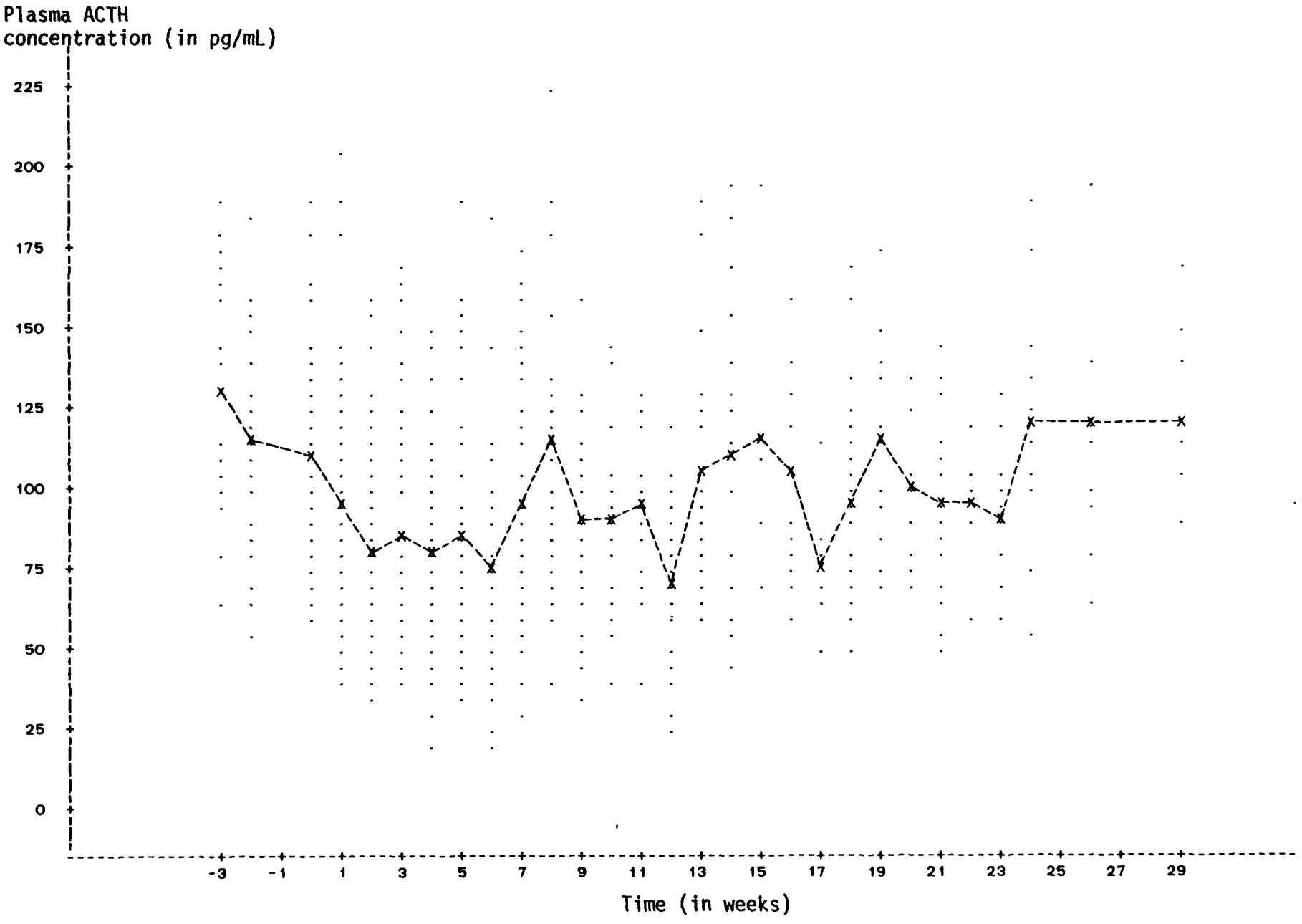


Figure 9. ACTH concentration data scatter diagram (exposure group).

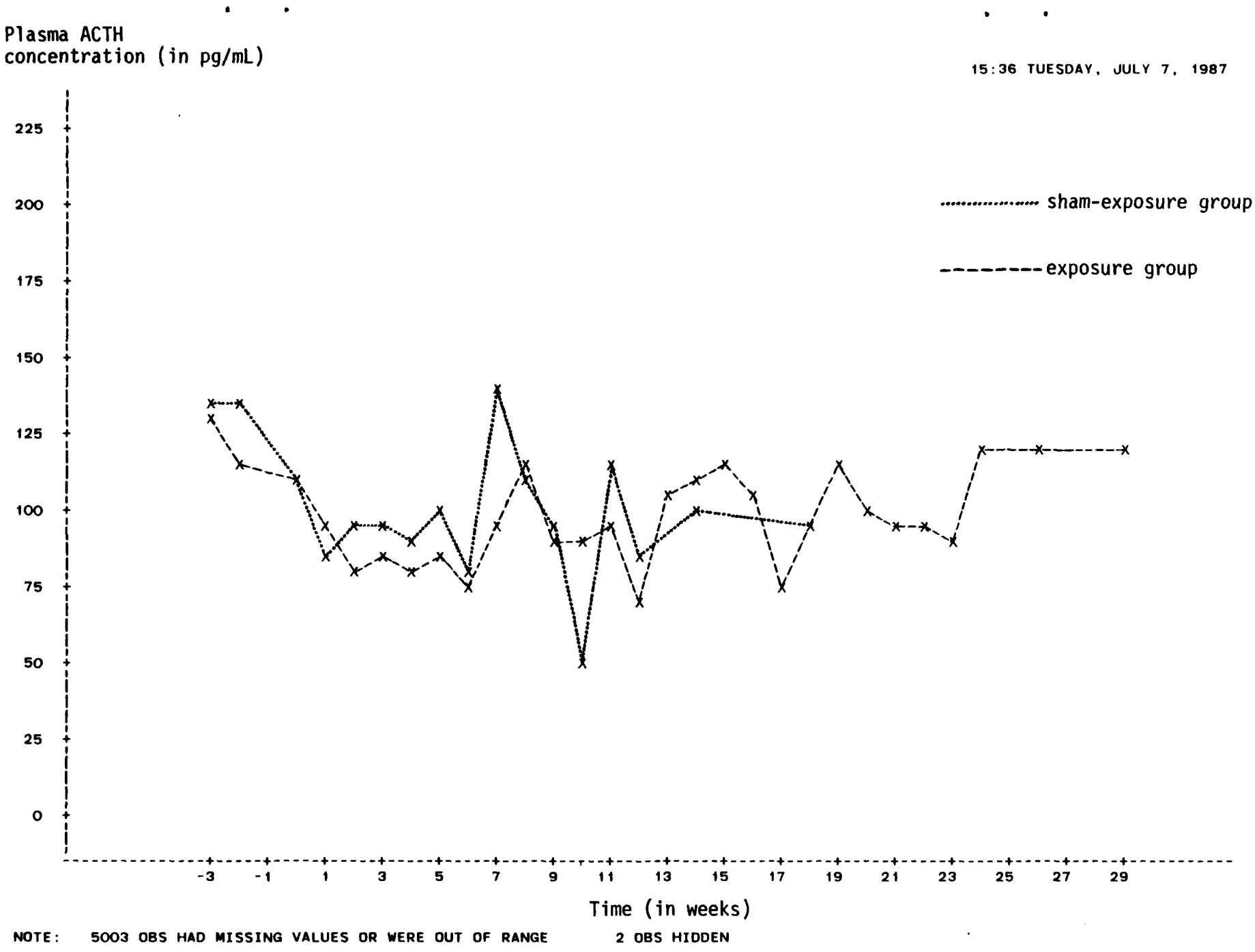


Figure 10. Mean plasma ACTH concentrations versus time.

exposure animals. At the experiment onset, the estimated resting value of ACTH was 99.5 pg/mL. Further analysis determined that, if there were any RFR-induced effects, they had to lie within a range of ± 7.37 pg/mL (between 92.1 to 106.9) from the resting value. Since resting ACTH concentrations between 50 and 150 pg/mL are considered normal in unstressed rats, then there was no indication that the long-term RFR exposure produced any stress as measured by plasma ACTH concentration.

Plasma corticosterone. Appendix G contains the data collected during the preradiation, radiation, and postradiation periods for both exposure and sham-exposure groups. Like ACTH, this data also displayed a high variance (for the same reasons as stated previously).

Figures 11 and 12 present the raw corticosterone data in scatter diagram form (the dotted lines pass through the mean response at each week). The plasma corticosterone data were somewhat better behaved than the ACTH data. Spikes occurred at weeks 7 and 12 in the sham-exposure animals (the spike at week 7 corresponds to that of ACTH at week 7 and both were probably due to the same cause), and at weeks 17 and 22 in the exposure animals.

Once again, both plots appeared to be essentially the same within experimental error; additionally, the RFR-exposed animals had corticosterone concentrations below that of their sham-exposed counterparts for most of the study (Fig. 13). This was an indication that 435-MHz RFR exposure did not increase the resting value of plasma corticosterone.

The corticosterone statistical analysis is detailed in the latter half of Appendix B. The procedure applied to the data was identical to that applied to plasma ACTH data and the result obtained was similar. Therefore, 435-MHz RFR was not found to increase resting plasma corticosterone concentration in the exposure group when compared to the sham-exposure group. In the sham-exposure group, plasma corticosterone concentrations rose from a level of approximately 13.6 μg/dL at the "exposure" onset to approximately 17.1 μg/dL at the "exposure" termination. Neither of these values was typical of corticosterone concentration in stressed rats. In the exposure group, plasma corticosterone concentrations started at approximately 13.6 μg/dL at exposure onset, dropped to approximately 10.3 μg/dL at week 9 of the radiation period, and then rose to approximately 17.9 μg/dL at the exposure conclusion. These values were also atypical of plasma corticosterone concentration in stressed animals. Further analysis indicated that, if there were any RFR-induced effects on plasma

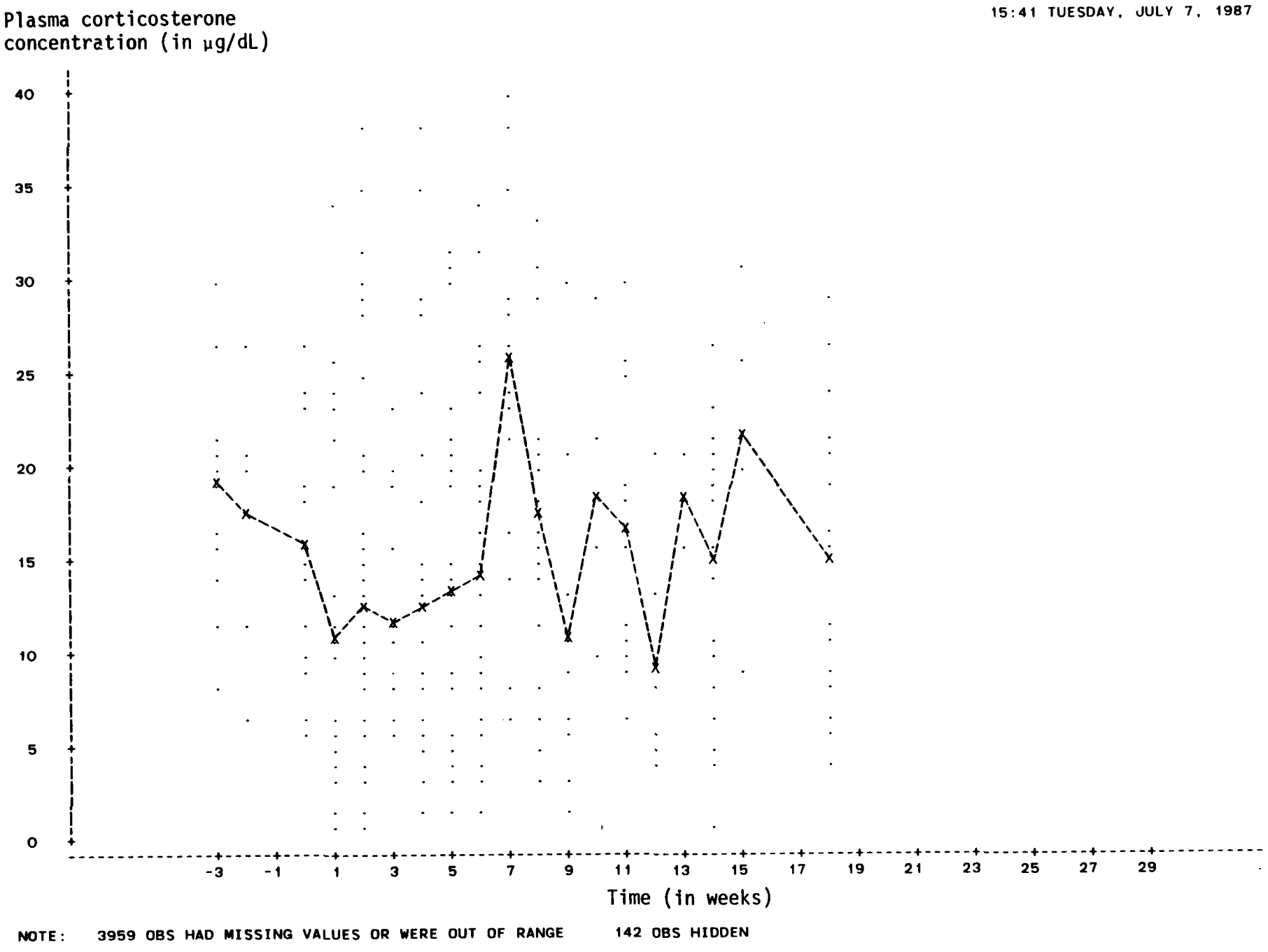


Figure 11. Corticosterone concentration data scatter diagram (sham-exposure group).

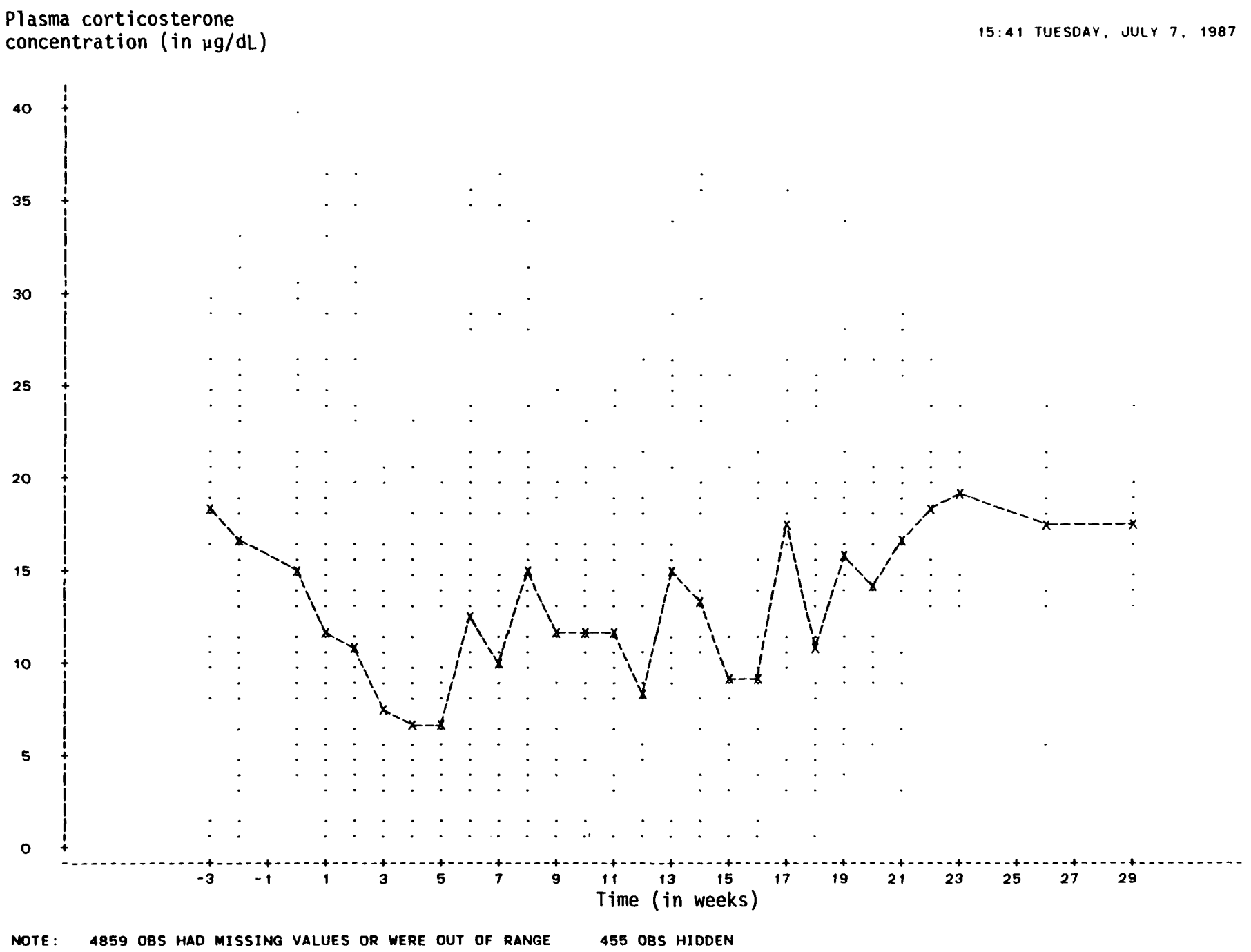


Figure 12. Corticosterone concentration data scatter diagram (exposure group).

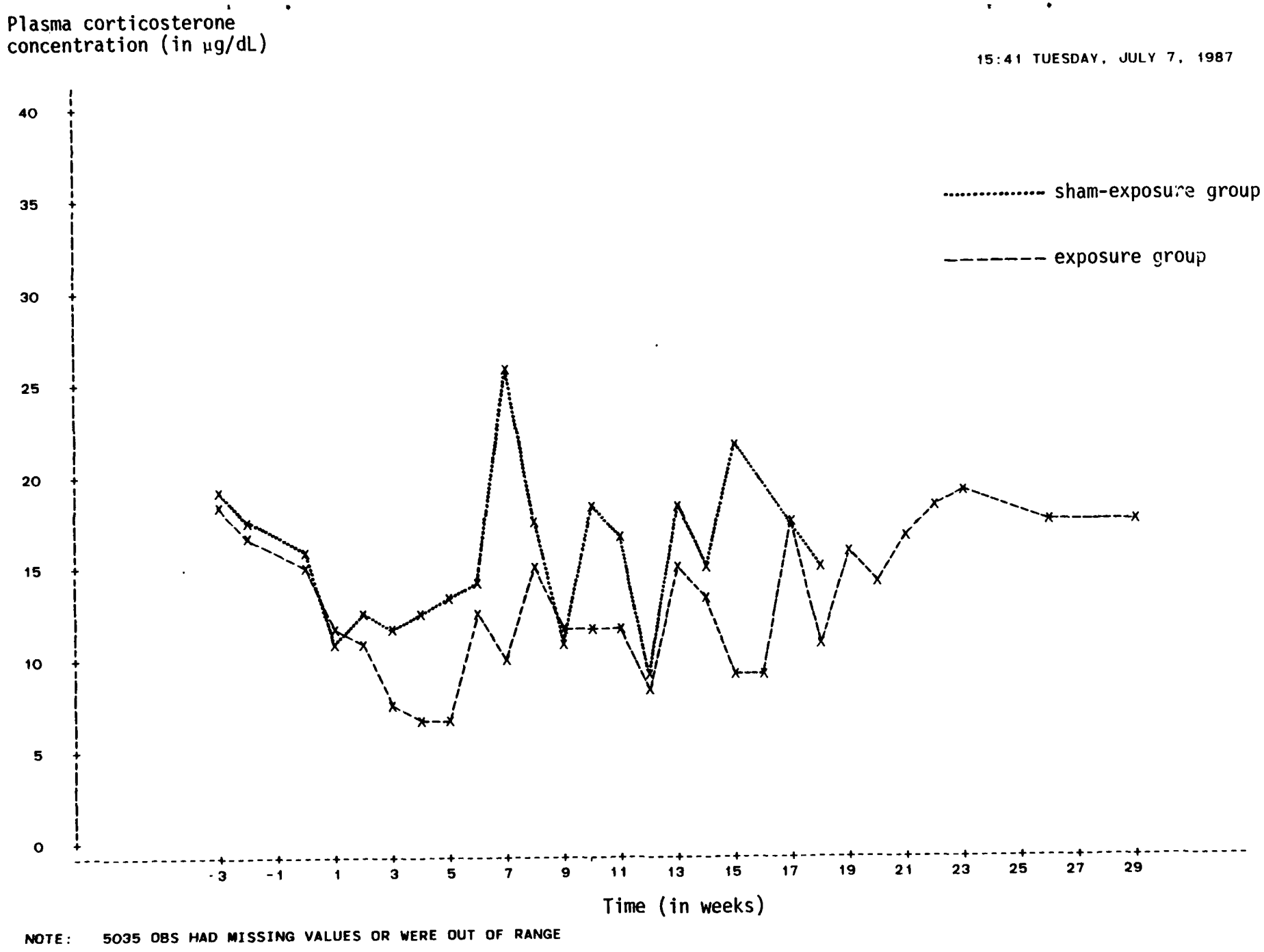


Figure 13. Mean plasma corticosterone concentrations versus time.

corticosterone concentration, they had to lie within a range of $\pm$ 1.31 μg/dL from the resting value. Since resting corticosterone concentrations below 25 μg/dL are considered normal in unstressed rats, then there was no indication that the long-term RFR exposure group had an elevated mean resting value of plasma corticosterone. Therefore, 435-MHz RFR did not appear to induce any stress in the exposure group, as measured by the concentration of plasma corticosterone.

IV. DISCUSSION

Stress has been described as the "non-specific response of an organism to any demand" [30,31]. Both external and internal stresses evoke increased ACTH and corticosterone secretion in the chronically cannulated rat [32].

Evaluation of the adrenocortical system in the rat depended on accurate determinations of plasma ACTH and plasma corticosterone. Values of basal levels of these hormones varied considerably among laboratories and depended also on the time when they were measured. The differences resulted from the increased sensitivity of new assays permitting measurement of very low concentrations of ACTH and corticosterone that were not possible a few years ago. Conditions under which animals were maintained during blood sampling were the source of other variations in resting levels of plasma hormones. Acute elevations of plasma ACTH and plasma corticosterone due to handling may mask experimentally induced stimulation of the adrenocortical system. The cannulation technique permitted repeated use of the same, unanesthetized, resting, and undisturbed rat for blood sampling and hormone determination, thereby minimizing the amount of handling needed to obtain a blood sample.

There are few studies concerned with investigating the effects of long-lasting stress on the pituitary-adrenocortical axis. For this reason, the work of Burchfield et al. [33] was of particular interest. Their rats received chronic or repeated acute cold stress during a period of up to 3 months. The authors found that chronically stressed rats had elevated resting plasma corticosterone levels, as much as seen in the control animals during an acute stress. On the other hand, plasma ACTH levels remained unchanged. Thus, chronic stress lasting 3 months increased the plasma level of corticosterone in undisturbed, resting rats from a value of 8-12 μg/dL to 23 μg/dL, but left the resting plasma ACTH level unchanged. The same experiments demonstrated also that longer stress duration led to higher plasma corticosterone levels. Sakellaris and Vernicos-Danellis [34] found that after adaptation to chronic stresses, and despite continued exposure to the stressor, the pituitary-adrenal system regulates plasma corticosterone concentration at the prestress levels. The pituitary secretion returned to control value after the adaptation to stress was completed.

The high sensitivity of the brain-pituitary-adrenal gland system requires only remote sampling of the blood. The repeated sampling of blood from the

same cannulated rat provided us with reliable information regarding resting patterns of ACTH and corticosterone secretion as well as any increases induced by a long-term, low-level RFR exposure. In other words, it seemed likely that even the smallest environmental perturbation, such as low-level RFR, would have been easily detectable if it has any influence on the brain-pituitary-adrenal axis. Although relocation of a rat from the cage into the sampling box 30 min before blood sampling slightly disturbed the environment of the rat, such a perturbation did not alter resting plasma ACTH and plasma corticosterone levels (waiting period and adaptation of the rat to the sampling box).

Short-term exposure to low-level RFR does not change plasma corticosterone levels of exposed rats [35,36] although Guy and associates [37] found an elevation of plasma corticosterone the first time the blood was sampled from RFR-exposed rats in their long-term study. In the same study, plasma corticosterone returned to resting control levels throughout the remaining 2-year period. It was possible that the early increase in plasma corticosterone of RFR-exposed rats was the consequence of excitement resulting from the animal's early exposure to the blood sampling process. Similar early increases in plasma corticosterone have been observed [32] in which the animals were not fully preconditioned to blood sampling boxes.

As far as we could determine, there is no information available regarding the effect of short-term, low-level RFR on plasma ACTH levels. This information would permit an evaluation of the pituitary-adrenal axis as a response to this environmental condition.

Both plasma ACTH and plasma corticosterone are sensitive indicators of various types of environmental stress in mammalian systems. In stressful situations, the plasma concentrations of both hormones increase, and the increase observed is often a function of stress intensity and duration. For example, previous studies [33] have demonstrated that long-term cold stress induced in rats a several-fold increase in resting corticosterone concentrations without a corresponding increase in ACTH concentrations. Johnson et al. [37] reported that a 2450-MHz pulsed-wave RFR environment may have been minimally stressful to rats over a 2-year exposure duration. Their conclusion was in part based on an elevation of plasma corticosterone levels detected during their first assessment period, followed by a return to basal levels for the remainder of the exposure, as well as a decrease in open-field behavior in the exposure group during the first assessment period.

Our results indicated that a 435-MHz pulsed-wave RFR environment did not increase either resting ACTH or resting corticosterone concentrations in rats. Further, a statistical analysis of the data indicated that if there were any RFR-induced effects on resting plasma hormone concentrations, they would lay within a range of $\pm$ 7.4 pg/mL from an estimated resting concentration of 99.5 pg/mL in ACTH; $\pm$ 1.3 μg/dL from an estimated resting concentration of 13.6 μg/dL in corticosterone. These values are not typical of rats exposed to stress. Therefore, this study concludes that a 1.0 mW/cm^2 435-MHz pulsed-wave (1.0-μs pulse width, 1-kHz pulse rate) RFR environment did not induce any detectable increase in stress, as measured by resting ACTH and corticosterone concentrations, in the exposure group of 87 cannulated male Sprague-Dawley rats when compared to a sham-exposure group of 65 cannulated male Sprague-Dawley rats.

V. REFERENCES

1. Toler, J., Bonasera, S., and Popovic, V. Long-term bioeffects of 435-MHz radiofrequency radiation on selected blood-borne endpoints in cannulated rats. Volume 1: Engineering considerations. Report USAFSAM-TR-86-4, September 1986.

2. Popovic, V., Toler, J., Bonasera, S., Popovic, P., Honeycutt, C., and Sgoutas, D. Long-term bioeffects of 435-MHz radiofrequency radiation on selected blood-borne endpoints in cannulated rats. Volume 3. Plasma prolactin. USAFSAM-87-6, June 1987.

3. Hodges, J.R., and Mitchley, S. The effect of training on release of corticotropin in response to minor stressful procedures in the rat. J Endocr 47:253-254 (1970).

4. Hennessy, M.B., Heybach, J.P., Vernikos, J., and Levine, S. Plasma corticosterone concentrations sensitively reflect levels of stimulus intensity in the rat. Physiol Behav 22:821-825 (1979).

5. Hennessy, J.W., and Levine, S. Stress, arousal and the pituitary-adrenal system: a psychoendocrine hypothesis. Prog Psychobiol 8:133-177 (1979).

6. File, S. The rat corticosterone response: habituation and modification by chlordiazepoxide. Physiol Behav 29:91-95 (1982).

7. Seggie, J.A., and Brown, G.M. Stress response patterns of plasma corticosterone, prolactin, and growth hormone in the rat, following handling or exposure to novel environment. Can J Physiol Pharmacol 53:629-637 (1975).

8. Hennessy, J.W., Levin, R., and Levire S. Influence of riential factors and gonadal hormones on pituitary-adrenal responses of the mouse to novelty and electric shock. J Physiol Psychol 91:770-777 (1977).

9. Baron, S., and Brush, F.R. Effects of acute and chronic restraint and estrus cycle on pituitary-adrenal function in the rat. Hormones Behav 12:218-224 (1979).

10. Barton, R.N., and Passingham, B.J. Early responses to hemorrhage in the conscious rat: Effects of corticosterone. Am J Physiol (Regulatory Integrative Comp Physiol 12) 243:R416-R423 (1982).

11. Bassett, J.R., Cairncross, K.D., and King, M.G. Parameters of novelty, shock predictability and response contingency to corticosterone release in the rat. Physiol Behav 10:901-907 (1973).

12. Fagin, K.D., Shinsako, J., and Dallman, M.F. Effects of housing and chronic cannulation on plasma ACTH and corticosterone in the rat. Am J Physiol (Endocrinol Metab 8) 245:E515-E520 (1983).

13. Ou, L.C., and Tenney S.M. Adrenocortical function in rats chronically exposed to high altitude. J Appl Physiol: Respirat Environ Exercise Physiol 47:1185-1187 (1979).

14. Raff, H., and Fagin, K.D. Measurement of hormones and blood gases during hypoxia in conscious cannulated rats. J Appl Physiol: Respirat Environ Exercise Physiol 56:1426-1430 (1984).

15. Knigge, K.M., Penrod, C.H., and Schindler, J. In vitro and vivo adrenal corticosterone secretion following stress. Am J Physiol 196:579-582 (1959).

16. Gale, G.C., Hayward, J.S., Green, W.L., Wu, Sy, Schiller, H.J., and Jackson I. Endocrine responses to acute cold in man. Fed Proc 34:301 (1975).

17. Cam, G.R., and Bassett J.R. The effects of acute nicotine administration on plasma levels of the thyroid hormones and corticosterone in the rat. Pharmacol Biochem Behav 19:559-561 (1983).

18. Guillemin, R., Dean, W.E., and Liebelt, R.A. Nychthemeral variations in plasma-free corticosteroid levels of the rat. Proc Soc Exp Biol Med 101:394-395 (1959).

19. Szafarczyk, A., Hery, M., Laplante, E., Ixart, G., Assenmacher, I., and Kordan, C. Temporal relationships between the circadian rhythmicity in plasma levels of pituitary hormones and in hypothalamic concentration of releasing factors. Neuroendocrinology 30:369-376 (1980).

20. Ader, R. Effects of early experience and differential housing on behavior and susceptibility to gastric erosion in the rat. J Comp Physiol Psychol 60:233-238 (1965).

21. Hatch, A.M., Wiberg, G.S., Zawidzka, Z., Cann, M., Airth, J.M., and Grice, H.C. Isolation syndrome in the rat. Toxicol Appl Pharmacol 7:737-745 (1965).

22. Lovely, R.H., Pagano, R.R., and Paolino, R.M. Shuttle-box-avoidance performance and basal corticosterone levels as a function of duration of individual housing in rats. J Comp Physiol Psychol 81:331-335 (1972).

23. Toler, J., Freedman, D., and Johnson, R. Prototype circular parallel plate facility for chronically exposing large rodent populations to 435-MHz RFR. Georgia Tech Final Report on Project A-2392, Contract No. F33615-78-D-0617 January 1980.

24. DHEW Publication (NIH) 78-23. Guide for the Care and Use of Laboratory Animals. Revised 1978.

25. Popovic, V., and Popovic, P. Permanent cannulation of aorta and vena cava in rats and ground squirrels. J Appl Physiol 15:727-728 (1960).

26. Popovic, V., Kent, K.M., and Popovic P. Technique of permanent cannulation of the right ventricle in rats and in ground squirrels. Proc Soc Exp Biol Med 113:599-602 (1963).

27. Engberg, A.A. A technique for repeated renal clearance measurement in undisturbed rats. Acta Physiol Scand 75:170-175 (1969).

28. Berson, S.A., and Yalow, R.S. Radioimmunoassay of ACTH in plasma. J Clin Invest 47:2725 (1968).

29. Donohue, J., and Sgoutas D. Improved radioimmunoassay of plasma cortisol. Clin Chem 21:770 (1975).

30. Selye, H. "The Physiology and Pathology of Exposure to Stress." Montreal: Inc. Medical Publishers, 1950.

31. Selye, H. "Stress and Aerospace Medicine." Aerospace Med 44:190-193 (1973).

32. Toler, J., and Popovic, V. Methods and approaches for studying biological effects of radiofrequency radiation. An overview. Proc Electromagnetic Compatibility, Zurich, 87-92, 1983.

33. Burchfield, S.R., Woods, S.C., and Elich M.S. Pituitary adrenocortical response to chronic intermittent stress. Physiol Behav 24(2):297-302 (1980).

34. Sakellaris, P.C., and Vernikos-Danellis, J. Increased rate of response of the pituitary-adrenal system in rats adapted to chronic stress. Endocrinology 97:597-602 (1975).

35. Lovely, R.H., Myers, D.E., and Guy, A.W. Irradiation of rats by 918 MHz microwaves at 2.5 mW/cm^2: Delineating the dose-response relationship. Radio Sci 12:139-146 (1977).

36. Johnson, R.B., Mizumori, S., Lovely, R.H., and Guy, A.W. Adaptation to microwave exposure as a function of power density and ambient temperatures in the rat. In Electromagnetic fields in biological systems. Abstracts 1978 IMPI/URSI Symposium, Ottawa Canada, 27-30 June.

37. Johnson, R.B., Spackman, D., Crowley, J., Thompson, D., Chou, C.K., Kunz, L.L., and Guy, A.W. Effects of long-term low-level radiofrequency radiation exposure on rats. Vol 4. Open-field behavior and corticosterone. Report USAFSAM-TR-83-42, December 1983.

APPENDIX A

RAW ACTH DATA SPREADSHEETS

ACTH – Controls I

Rat #	Group	-3WK	-2WK	0WK	TIME 1WK	2WK	3WK	4WK	5WK	6WK	7WK	8WK	9WK	10WK	11WK	12WK	13WK	14WK	15WK	16WK	17WK	18WK	19WK	20WK	21WK	22WK	23WK	24WK	+2	+5
1	C				69	92	92	117		42		66	44			163		70				169								
2			187	80	58	135	137	94		55		73	57			59		55												
3			141	93	132	102	102	87																						
4			60	66	74	86	86	109		63		82	121			76		46				143								
5			107	160	82	82	84	93		68		68	123	50		135														
6			145	121	42	106	109	110		65		96				79		96				191								
7				127	122	101	110	128		76																				
8	C			90	51	98	132	98				85				70		94				137								
9	E			181	54	52	57					87	89					132				103								
10				160		98	96					60	64			66		171				78								
11				45		101	94				74		60									93								
12				191		81																								
13				102		77	60	82	40				91			80		103				77								

ACTH – Controls II

Rat #	Group	-3WK	-2WK	0WK	TIME 1WK	2WK	3WK	4WK	5WK	6WK	7WK	8WK	9WK	10WK	11WK	12WK	13WK	14WK	15WK	16WK	17WK	18WK	19WK	20WK	21WK	22WK	23WK	24WK	+2	+5
14				171	68	69	74	54	42		88		93					161												
15		137		61		67	72	76	76		135		68					122												
16	EC1			80		52	97	56	92		121		89					160												
17	EC2	140		96	102																									
18		121		47	137	127		102		77								144												
19					77	116		115		54					124															
20					65	92		102		111					131			161												
21					57	93		123		58																				
22					64	80		91		98								170												
23					65	97		139										124												
24					41	86				92								101												
25					60	106		117										103												
26					58	117		106		81								127												

↑ C
↓ S

ACTH – Control III

Rat #	Group	-3WK	-2WK	0WK	TIME 1WK	2WK	3WK	4WK	5WK	6WK	7WK	8WK	9WK	10WK	11WK	12WK	13WK	14WK	15WK	16WK	17WK	18WK	19WK	20WK	21WK	22WK	23WK	24WK	+2	+5
27					153	100		87		66																				
28					189	75		96		92					150															
29					114	87		71		50					73															
30					193	95		68		63					96															
31					115	80				31					66															
32					139	95		91		66					116															
33					146	128		64		56					166															
34					122	135				51					115															
35					99	81		68		33					123															
36					138	89		80		67					117															
37					140	56		59		57																				
38					118	79		65		68					142															
39	DV					78			35	147	71	170																		

ACTH – Control IV

Rat #	Group	-3WK	-2WK	0WK	TIME 1WK	2WK	3WK	4WK	5WK	6WK	7WK	8WK	9WK	10WK	11WK	12WK	13WK	14WK	15WK	16WK	17WK	18WK	19WK	20WK	21WK	22WK	23WK	24WK	+2	+5
40						90			117	265	142		169																	
41						59			101	139	174																			
42						89			225	161	159																			
43						63			152	66	101	156																		
44						84			74	117	287	164																		
45						74			80	139	123	187																		
46						70			126	80	167																			
47						70			183	59	299	135																		
48						71			99	132	179		150																	
49						207			94	157																				
50						84			96													117								
51						93			99													91								
52						83			123													88								

ACTH – Control V

Rat #	Group	-3WK	-2WK	0WK	TIME 1WK	2WK	3WK	4WK	5WK	6WK	7WK	8WK	9WK	10WK	11WK	12WK	13WK	14WK	15WK	16WK	17WK	18WK	19WK	20WK	21WK	22WK	23WK	24WK	+2	+5
53						76			160									114				116								
54						48			90									127				129								
55						81			138									131												
56						76			118									131				48								
57						65			84									40				104								
58						202			146									76				70								
59						96			133									116				47								
60						80			62																					
61									44									33				49								
62						81			47																					
63						161			70									140				66								
64						102			109																					
65						117			45									121				108								

ACTH – Microwave I

Rat #	Group	-3WK	-2WK	0WK	TIME 1WK	2WK	3WK	4WK	5WK	6WK	7WK	8WK	9WK	10WK	11WK	12WK	13WK	14WK	15WK	16WK	17WK	18WK	19WK	20WK	21WK	22WK	23WK	24WK	+2	+5
1	A	82	140	116	92	50	52	76	48	42	76	84	←																96	96
2		80	102	78	89	38	46	83	58	56	86	120																		
3		173	155	107		35	52																							
4		140	183	103	146	48	39																							
5		80	64	82	83	54	48	61	38	35	126		←																81	102
6		66	113	88	97	129	62	89	53		124																			
7	double Lan A&B	140	113	114	65	50	52	83	37	42	149																			
8		110	90	77	82	124	46	101	54	56	147		←																93	40
9		100	68	81		52																								
10		114	114	132	143	43	39	39																						
11		94	68	122	38	55	77	68	47	36	106																			
12			54	101	88	48	52	96	43	50		132	←																140	91
13			101	83	74	81					42																			

A ↓ A dr AA

ACTH - M II

Rat #	Group	-3WK	-2WK	0WK	TIME 1WK	2WK	3WK	4WK	5WK	6WK	7WK	8WK	9WK	10WK	11WK	12WK	13WK	14WK	15WK	16WK	17WK	18WK	19WK	20WK	21WK	22WK	23WK	24WK	+2	+5
14			87	95	67																									
15			128	94	110	45	73	78	50	46	148			66			80	80			63	88							89	100
16			118	136	180	48	71	79	65	53	161																			
17				82		38	74										99				45	69							61	105
18																														
19																														
20																														
21																														
22	B↓		104	91	52	310	103	109				122		55		77		70			68	69	81						102	68
23			120	158	52	146	121	106												141										
24		164	110	180	41	155	79	83	84	87	84		81	90		68		80												
25		99	127	147	65	123	149	104		66			87	120		100		140												
26			160	181	111	113				79	80	64		116		98		102			80									

ACTH - M III

Rat #	Group	-3WK	-2WK	0WK	TIME 1WK	2WK	3WK	4WK	5WK	6WK	7WK	8WK	9WK	10WK	11WK	12WK	13WK	14WK	15WK	16WK	17WK	18WK	19WK	20WK	21WK	22WK	23WK	24WK	+2	+5
27		181	102	99	90	110		83		187		224				62		80												
28		107	140	166	75	107	122	94		90		88						16				124							102	68
29		168	148	145	64	107	125	105		116		134		96		104		186				68							76	74
30		160	161	124	78	130	106	150	136	150																				
31			104	98	105	126	60	112	116	94																				
32			151	65	119	87	74	93	146					91		99		55		60		101	120							
33	B↓		89	167	47	75	73	137	144					86		120		81		88		60							102	126
34	D↓		80	124	124		113	68			86		33					171												
35			116	59	135	85	108	108			107		51		76	75		127												
36		190	161	64	100		61	93	106	60	80	61	71																	
37			92	80	85	66	116	52	58		135		81																	
38			64	69	93		160				60																			
39			101	103	61		163	114	71		64							193											84	91

ACTH - MIV

Rat #	Group	-3WK	-2WK	0WK	TIME 1WK	2WK	3WK	4WK	5WK	6WK	7WK	8WK	9WK	10WK	11WK	12WK	13WK	14WK	15WK	16WK	17WK	18WK	19WK	20WK	21WK	22WK	23WK	24WK	+2	+5
40			107	89	121		134	112	51				75						104			105	69	←					99	116
41			147	166	129		169				107																			
42			65	98	124	78	140	112	61	90	78		116									49								240
43	D ↑			107	120		105				76		109						70			66		←					102	64	D ↑
44	E ↓		117	140	107	62	62	91	83	79	89																			E ↓
45			89	90	105	51	85	69	63	49	49																			
46			120	85	110	52		91	72	91	83											80		←					80	81
47				64	120	93		81																						
48			113	95	112	63		76		143					69		91					112		←					126	70	313
49			125	102	109	109		103		79	86		57		91		75													
50			161	114	98	94		79		50			64		75		68		90		84	121		←					135	48	
51			140	145	101	120	63	147	76						84															
52				127	78	89	104	145	64	74	94				64		61		112			61		←					107	108	319

ACTH - MV

Rat #	Group	-3WK	-2WK	0WK	TIME 1WK	2WK	3WK	4WK	5WK	6WK	7WK	8WK	9WK	10WK	11WK	12WK	13WK	14WK	15WK	16WK	17WK	18WK	19WK	20WK	21WK	22WK	23WK	24WK	+2	+5
53					108	100		59		109																				
54					141	56		69		56																				
55					77	92		126		101					92															
56		143	109	97	76	100		81		50					105				69						104	90	130	146	141	170
57			156	160	68	100		93		66					125				121						144	105	93	183	142	148
58		162	117	120	74	89				79					99				140						146	120	60	177	122	141
59					57	73		68		56					123				91								88	134	80	116
60					98	98		107		49					92				75								80	56	118	90
61					122	75		59		40					99				83						89		79	98		
62					54	115		74		80					132				129								80	117		
63					70	87		92		68					121				161						55		132	125	195	88
64	F ↑				93	88		88		92					104				80								61	103	96	107
65	↓					84			125																		119	77		

ACTH – M VI

Rat #	Group	-3wk	-2wk	0wk	1wk	2wk	3wk	4wk	5wk	6wk	7wk	8wk	9wk	10wk	11wk	12wk	13wk	14wk	15wk	16wk	17wk	18wk	19wk	20wk	21wk	22wk	23wk	24wk	+2	+5
66	M ↓ te					63			92		116	188				78	93	58				52	133							136	
67						67			94		83	113				67	94	78				113	162	133						129	
68						57			50		120	93				81	84	40					95							120	
69						96			161		84	88				90	151	78						69						139	
70						128			80		83	129				72	188	132					100	96						64	
71						75			120		97	126				60	180	14					116	119						93	
72						158			105		96	39				66	115						73	149						115	
73						126			192		163	110				25	118	57					88	134						87	
74						59			79		90	82				93	65	115					115	139						118	
75									53		142	113				63	68	154					65	173						116	
76						62			103		91	88					130	99					106	122						139	90
77						63			97		139	122					124	47					169	117						102	114
78	OP ↓					42	74	58	93		28	74	77																	

M ↓ ; ↕ D, DI

ACTH – M VII

Rat #	Group	-3wk	-2wk	0wk	1wk	2wk	3wk	4wk	5wk	6wk	7wk	8wk	9wk	10wk	11wk	12wk	13wk	14wk	15wk	16wk	17wk	18wk	19wk	20wk	21wk	22wk	23wk	24wk	+2	+5
79					67	77	58	28	41	65	28	74	77	88		44														
80					67	128	59	59	147	91	60	154	123	120		101														
81					63	78	67	50	35	101	72	190	89	97		27														
82					65	81	51	123	150	79	79	115	160	70		40														
83					42	80	106	64	93	101	75	179	126	107		46														
84					69	98	02	95	19		89	115	130	40		28														
85					83	62	71	40	88	74	74	71	69	60		70														
86					104	74	72	50	154	20	122	116	102	79		49														
87					141	69	53	20	50	97	53	101	45			40														
88					112	70	56	20	88	62	49	221	106	85		87														
89					192	74	59	40	121																					
90					206	80	141	56																						
91																														

APPENDIX B

STATISTICAL METHODOLOGY

APPENDIX B

STATISTICAL METHODOLOGY

The balanced design of this experiment (requiring that 25 animals from each 100 animal group be sampled once every 3 weeks for stress hormones) should have produced data easily tested by balanced, 2-way analysis of variance (ANOVA) statistics with 12 levels of factor A (time) and 2 levels of factor B (RF radiation). However, data collection did not proceed according to protocol in that, in numerous cases, samples were collected at odd intervals (invalidating the orthogonality of the design) and the number of samples taken per week varied above and below the 25 animal mark (unbalancing the design). These two factors combined to lower the power of ANOVA statistics (power being defined as the ability to reject the null hypothesis given the null hypothesis should be rejected) trying to test the model:

$$y_{ijk} = \mu + \tau_i + \beta_j + \tau\beta_{ij} + \varepsilon_{ijk}, \qquad \text{(B-1)}$$

where y_{ijk} = hormone concentration (response),
μ = the normal hormone resting concentration,
τ_i = the change in hormone resting concentration induced by RFR,
β_j = the change in hormone resting concentration induced by time,
$\tau\beta_{ij}$ = the change in hormone resting concentration induced by the interaction between RFR and time, and
ε_{ijk} = noise within the system (sampling and assaying errors)

for the following hypotheses:

$$H_0: \tau_0 = \tau_1 = 0, \qquad \text{(B-2)}$$
$$H_1: \tau_0 \text{ or } \tau_1 \neq 0 \text{ (RFR-induced effects)},$$

$$H_0: \beta_1 = \beta_2 = \ldots = \beta_{12} = 0, \qquad \text{(B-3)}$$
$$H_1: \text{at least one } \beta_j \neq 0 \text{ (time-induced effects)},$$

$$H_0: \tau\beta_{ij} = 0, \text{ and} \qquad \text{(B-4)}$$
$$H_1: \text{at least one } \tau\beta_{ij} \neq 0 \text{ (interaction between RFR and time)}.$$

However, examination of the collected data suggested an alternative approach in that the data resembled what might have been collected in an unplanned experiment monitoring over time the operation (in this case, characterized by resting animal hormone concentrations) of an established RF radiation facility. Data of this type are often successfully treated by employing linear regression techniques to develop, build, and test a linear (or intrinsically linear) model whose parameters can be used to predict the system response at various treatment levels. Therefore, we decided to proceed with a regression approach to data analysis.

Plasma ACTH Statistical Analysis

Examination of the ACTH scatter diagrams of Figures 8 and 9 showed an essentially linear response (factoring out spikes displayed at weeks 7, 10, 8, 12, and 17) of plasma ACTH versus time. Therefore, the linear model to test for RFR-induced effects on the ACTH concentration would include a nonzero β_0 term (which provided an estimate of the mean hormone resting level at week 0), and an $\alpha_0 z$ term to test for any biasing of normal resting levels due to RFR. Since the line traced by the mean hormone values in the scatter diagrams was not completely straight, linear terms (β_1 and $\alpha_1 z$) were included in the model to adjust for a change in slope over time, and quadratic terms (β_{11} and $\alpha_{11} z$) were included in the model to adjust for any curvature in the response.

Therefore, the initial model became:

$$y = \beta_0 + \beta_1 x + \beta_{11} x^2 + \alpha_0 z + \alpha_1 zx + \alpha_{11} zx^2, \qquad \text{(B-5)}$$

where y = plasma ACTH concentration,

x = time (in weeks), and

z = a categorical variable with value 0 for animals in the sham-exposure group and value 1 for animals in the exposure group.

Raw data from the ACTH spreadsheet (included in Appendix A) were put on computer file, and a Statistical Analysis System (SAS) formatting program (included in Appendix C) was prepared to read the data file and perform the desired statistical tests on the model.

The first test identified terms within the general model which contributed the least toward forming a statistically significant regression. These

procedures were used in combination with an initial regression on the general model (not included) to evaluate the statistical significance of terms modelling the ACTH concentration time dependency and terms modelling the RFR-induced effects on ACTH concentration. Two types of model "building" procedures were employed: forward stepwise regression and maximum R^2 regression. Forward stepwise regression produced a "best" model (in that it minimized the number of model predictor variables) in which all the included terms were statistically significant at a level of 0.15. Maximum R^2 regression approached model building from a somewhat different perspective; it first built the "best" one-predictor model (in that it maximized R^2, the percentage of variation within the data explained by the regression), then built the "best" two-predictor model, the "best" three-predictor model, etc., until it arrived at the general model. All these tests indicated that the model which best fit the data was

$$y = \beta_0 + \beta_1 x + \beta_{11} x^2 + \alpha_1 xz, \qquad \text{(B-6)}$$

where

$$\beta_0 = 99.50,$$
$$\beta_1 = 1.79,$$
$$\beta_{11} = 0.12,$$
$$\alpha_1 = -0.68,$$

and the other variables were as defined previously. The outputs of both procedures are included in Appendix D. The absence of α_0 indicated that RFR did not produce a detectable effect on the intercept of the model, and therefore did not bias the ACTH concentration of the exposure group when compared to the sham-exposure group. Also, RFR did not produce a detectable effect on the curvature of the exposure group's ACTH concentration when compared to the sham-exposure group.

The sham-exposure group did display a difference, when compared to the exposure group, in the overall time response of hormones. In both groups, ACTH concentrations at the onset of exposure were somewhat high (106 pg/mL for sham-exposure animals, 108.1 pg/mL for exposure animals), dropped into the middle of the exposure duration (93.1 pg/mL at week 11 for sham-exposure animals, 87.1 pg/mL at week 14 for exposure animals), and then rose at exposure termination (151.6 pg/mL 5 weeks after exposure termination for sham-exposure animals, 131.8 pg/mL 5 weeks after exposure termination for exposure animals). The differences

in predicted ACTH concentration at the end of the exposure were enlightening. Basically, the trend (modelled by the negative $\alpha_1 xz$ term) was that ACTH concentrations in the exposure group remained lower than ACTH concentrations in the sham-exposure group for the exposure duration. Both ranges (93.1 to 151.6 pg/mL in sham-exposure animals, 87.1 to 131.8 pg/mL in exposure animals) were still well within the normal range of plasma ACTH in non-stressed male Sprague-Dawley rats (approximately 75-175 pg/mL). Therefore, these results indicated that 435-MHz RFR did not induce an elevation of ACTH concentration in the exposure group.

The just mentioned conclusions could be accepted only after the assumptions underlying the model used to draw the conclusions were verified. These assumptions included no model lack-of-fit, NID(0, σ^2) residual distribution, and no model multicollinearity.

Since multiple observations of ACTH concentration were taken for the weeks containing data, it was possible to perform a model lack-of-fit test on the regression. The lack-of-fit test involved breaking the sum-of-squares error from the regression into two components: sum-of-squares pure error, representing the actual variation due to the sampling and assaying process; and sum-of-squares lack-of-fit, representing the variation due to the difference between the mean value at one week when compared to the fitted value at the same week. A test statistic was then computed comparing the sum-of-squares lack-of-fit to the sum-of-squares pure error; sufficiently high values of the test statistic indicated model lack-of-fit.

Sum-of-squares error was obtained from the ANOVA table produced in the regression procedure output. Sum-of-squares pure error was obtained by analyzing the experiment from 2-way, fixed effects ANOVA viewpoint. The sum-of-squares lack-of-fit was then computed from the difference of sum-of-squares error minus sum-of-squares pure error. Calculations to compute the critical value F_0 are detailed in Appendix E.

The computed test statistic F_0 exceeded the critical value, thereby indicating significant lack-of-fit. Normally, this result would be somewhat disturbing since it would require refitting the model using transformed rather than raw data values. In fact, transformation of the dependent variable y was definitely undesirable, since the residual plots indicated that the residuals of y (using the revised model) conformed to the NID(0, σ^2) requirement. Additionally, the transformation of the predictor variables x and x^2 to yield a

model displaying no lack-of-fit, although theoretically possible, would be a long and time-consuming process.

Fortunately, the experimental design helped compensate for the model lack-of-fit deficiency. First of all, the lack-of-fit was comparatively small. Under optimal conditions (lack-of-fit statistically insignificant), both the mean square error and the mean square pure error estimate the population variance. If there is a lack-of-fit, the mean square pure error estimates the population variance, while the mean square error estimates the population variance plus a bias term. From the ANOVA (regression and GLM) tables, the tabulated values for MS_E and MS_{pe} were 1247.19 and 1132.31 respectively. These values corresponded to sample standard deviations of 35.32 pg/mL and 33.65 pg/mL. Thus, although the lack-of-fit was statistically significant, it was also practically insignificant. In other words, the development of an alternative model displaying no lack-of-fit would yield essentially (within 1 or 2%) the same results as the present model displaying lack-of-fit. Rather than identify an alternate model (which would not be that much better a predictive tool than the model currently being used), we decided to proceed with the stepwise model and modify the significance of the tests to compensate for model lack-of-fit. Thus, all α values are somewhat higher than they should be, and the confidence intervals established are somewhat wider than indicated in the appendix tables.

The next step in determining model accuracy involved examining the residual and partial residual plots to verify the least-squares regression assumption that the model errors were $NID(0,\sigma^2)$. Confirming this assumption defended the use of F tests to determine the statistical significance of the parameters, and validated the statistics which produced the tables listing confidence intervals of the ACTH concentrations. A number of residual plots suggested themselves immediately: residuals versus time, residuals versus predicted value of ACTH concentration, residuals versus animal case number, studentized residuals versus the above three, and partial residual plots corrected for the model terms β_0, β_1, β_{11}, and α_1. Since the order of each sample's assay was unavailable, there was no way to test for correlation using either the Durbin-Watson or runs test.

Examination of the residual plots yielded no discernible pattern in the distribution of the residuals, thus confirming the $NID(0,\sigma^2)$ hypothesis. However, four outliers at

animal #06	week 18	[ACTH] = 290 pg/mL
animal #40	week 16	[ACTH] = 265 pg/mL
animal #44	week 7	[ACTH] = 287 pg/mL
animal #47	week 7	[ACTH] = 299 pg/mL

were detected using the criterion of Cook's distance (each of the above observations had a Cook's D of approximately 1%, making their contribution to the model inappropriately large considering the size of the data set). These outliers were most likely the result of assaying or reporting error, and were considered sufficiently anomalous to be rejected from the data set. Residual plots were then regenerated (see Appendix F) and checked for patterns. The new residual plots in turn did not challenge the $\sim NID(0,\sigma^2)$ assumption inherent in least-squares model fitting.

To complete the analysis, diagnostics to check for model multicollinearity and correlation between the terms were employed. Examination of the listed condition numbers and matrix eigenvalues (being provided under separate cover) detected no troublesome values. This analysis indicated that the model did not display a significant degree of multicollinearity. Similarly, examination of the correlation matrix showed that correlation between the estimated values of β were all within tolerable limits. The highest degree of correlation was between the x and the x^2 term, which often occurs when using a polynomial model in linear regression.

For future reference, and for the sake of completeness, tables listing animal case number, observations (if taken) at each week, predicted value of ACTH concentration, standardized error of prediction, 95% confidence intervals on the mean value of the ACTH concentration, and residuals were prepared, as were tables containing animal case number, regular and studentized residual values, a graphical display of student residual values, and influence statistics (such as Cook's D). These tables were used to detect both outliers and influential data points in the ACTH data set.

To arrive at a conservative estimate of the minimum change due to RFR in resting ACTH concentrations which this protocol was capable of detecting, the value of the operating curve parameter Φ_B corresponding to the time factor (B) discussed at the beginning of the statistical methodology was calculated. This parameter was given by

$$\Phi_B^2 = \frac{naD^2}{2b\sigma^2} , \qquad \text{(B-7)}$$

where n = number of replications per cell = 40,
a = number of levels of factor A = 12,
b = number of levels of factor B = 2,
σ^2 = population variance, and
D^2 = detection threshold.

Substituting in values for a, b, n, and the MS_{pe} as an estimate of σ^2 provided an operating curve parameter of

$$\Phi_B = 0.3255\ D \qquad \text{(B-8)}$$

To obtain a value of Φ from the operating curve, the type I risk α and type II risk β were set to 0.05 and 0.10, respectively. Then, the value of Φ was read from the fixed effects ANOVA curve with $\nu_1 = 1$ and $\nu_2 = 936$. This value was

$$\Phi_B \doteq 2.4 \qquad \text{(B-9)}$$

Note that the numerator degrees of freedom, ν_1, and the denominator degrees of freedom ν_2, were calculated from the equations

$$\nu_1 = b-1, \text{ and} \qquad \text{(B-10)}$$
$$\nu_2 = ab(n-1). \qquad \text{(B-11)}$$

The detection level was therefore

$$D_B = 7.37 \text{ pg/mL}. \qquad \text{(B-12)}$$

Thus, this protocol conservatively was able to detect an increase in resting plasma ACTH concentrations of 7.37 pg/mL approximately 90% of the time.

Plasma Corticosterone Statistical Analysis

Examination of the corticosterone scatter diagrams of Figures 11 and 12 showed a situation similar to that of ACTH. The mean corticosterone values, excepting spikes at weeks 7, 12, 17 and 22, followed an essentially linear response over time. Therefore, the linear model

$$y = \beta_0 + \beta_1 x + \beta_{11} x^2 + \alpha_0 z + \alpha_1 zx + \alpha_{11} zx^2, \qquad \text{(B-13)}$$

where y = plasma corticosterone concentration,
x = time (in weeks), and
z = a categorical variable set to 0 for the sham-exposure animals and 1 for the exposure animals,

was again employed. Data from the corticosterone spreadsheet (included in Appendix G) were put into a new file and a second SAS formatting program (included in Appendix H) was prepared to analyze the data.

The model indicated by the forward and maximum R^2 stepwise regression procedures was

$$y = \beta_0 + \beta_1 x + \alpha_1 xz + \alpha_{11} x^2 z, \qquad \text{(B-14)}$$

where $\beta_0 = 13.55$,
$\beta_1 = 0.15$,
$\alpha_1 = -0.83$, and
$\alpha_{11} = 0.036$,

with the x, y, and z variables as defined previously. The outputs of both procedures are included in Appendix I. The absence of α_0 indicated that RFR did not induce a detectable change in the intercept of the model and therefore did not bias the corticosterone concentration of the exposure group when compared to the sham-exposure group.

In the case of the sham-exposure group, the resting plasma corticosterone concentration slowly rose during the exposure from a level of 13.55 μg/dL at the study onset to 17.08 μg/dL at week 24 of the exposure schedule. Both of these values were well within the normal resting range of plasma corticosterone in unstressed male Sprague-Dawley rats. The exposure group displayed a small amount of curvature when compared to the sham-exposure group, with corticosterone concentrations of 13.55 μg/dL at exposure onset, dropping to 10.32 μg/dL at week 9, and then rising to 17.93 μg/dL at week 24 of the exposure schedule. Once again, these values were well within the normal resting range of plasma corticosterone in unstressed male Sprague-Dawley rats. Therefore, from the above model, RFR did not induce an elevation of plasma corticosterone in the exposure group. The trend displayed in the data was that sham-exposure corticosterone concentrations gradually increased over time, the increase

remaining well within the boundaries of plasma corticosterone in unstressed rats. The exposure group's corticosterone levels tended to fall at the exposure onset, and consistently remained lower than those of the sham-exposure group until exposure termination, when the two group's concentrations were essentially equal. Although the two groups did not behave in an identical manner, there was no statistical basis to indicate that RFR induced a stress which manifested itself as a difference in plasma corticosterone concentration between the exposure and sham-exposure groups.

It was then necessary to check the model lack-of-fit, the residual distribution, and the amount of multicollinearity present in the model. First, the model was checked for lack-of-fit. Sum-of-squares error from the regression was 92991.99 with 1297 degrees of freedom and sum-of-squares pure error was 81974.68 with 1254 degrees of freedom. Therefore, sum-of-squares lack-of-fit was 11017.31 with 43 degrees of freedom, and F_0 was calculated to be 3.9195 (see Appendix J for details of this calculation).

As in ACTH, the model derived for corticosterone displayed a significant lack-of-fit. Transformation of the response variable y was undesirable since the residuals of y (generated with the given model predictors) conformed to the $NID(0,\sigma^2)$ hypothesis. Transformation of the x values, while possible, would remain a rather long and time-consuming process.

This problem was solved the same way as with ACTH. Since the lack-of-fit was comparatively small (with MS_E = 71.70 and MS_{pe} = 65.37 as estimates of the corticosterone variance), the difference in predicted responses between a model displaying insignificant lack-of-fit and this model (displaying significant but small lack-of-fit) would only amount to values differing from one another by 1 to 2%. Therefore, an analysis using the model that displayed some lack-of-fit was used. This lack-of-fit was then compensated for by altering the significance levels α for the conclusions drawn from the analysis.

Appendix K contains the residual plots generated from the data set once the outliers were removed. The plots all showed no discernible pattern, and thereby did not void the assumption that the model ε_is were distributed as normal random variables. Tests for independence were not possible since the order of each sample's assay was unavailable. Therefore, from the given residuals and data, there was no reason to reject the assumption that the residuals were distributed $NID(0,\sigma^2)$.

The outliers detected by Cook's distance and removed from the data set were as follows:

animal # 14	week 14	[cortico] = 38 μg/dL
animal # 23	week 4	[cortico] = 48 μg/dL
animal # 54	week 2	[cortico] = 49 μg/dL
animal # 55	week 2	[cortico] = 57 μg/dL
animal #124	week 2	[cortico] = 40 μg/dL
animal #125	week 1	[cortico] = 41 μg/dL
animal #143	week -3	[cortico] = 38 μg/dL
animal #178	week 10	[cortico] = 44 μg/dL

As was the case with ACTH, these outliers were most likely the result of assaying or recording errors, and were considered sufficiently anomalous to be rejected from the data set.

Examination of model multicollinearity diagnostics (provided under separate cover) did not indicate any significant model multicollinearity. The values for the condition indices were all within the [1,10] range considered tolerable. The highest correlation in the model (-0.7784) was between the parameters α_1, and α_{11}. This was not unexpected, since linear and quadratic terms often display a degree of correlation in polynomial regression situations. Additionally, this correlation was well within the $\pm$ 0.95 correlation criteria which often indicates multicollinearity problems. Therefore, we concluded that multicollinearity and correlation did not pose serious challenges to the validity of the obtained model.

For future reference, and for the sake of completeness, tables listing animal case number, observations (if taken) at each week, predicted value of corticosterone concentration, standardized error of prediction, 95% confidence intervals on the mean value of the corticosterone concentration, and residuals were prepared, as were tables containing animal case number, regular and studentized residual values, a graphical display of student residual values, and influence statistics (such as Cook's D). These tables were used to detect both outliers and influential data points in the corticosterone data set.

To arrive at a conservative estimate of the minimum change due to RFR in resting plasma corticosterone concentrations which this protocol was capable of detecting, a calculation was made for the value of the operating curve parameter

Φ_B corresponding to the RFR factor discussed at the beginning of this Section. Using the equation

$$\Phi_B^2 = \frac{naD^2}{2b\sigma^2}, \tag{B-15}$$

where n = number of replications per cell = 40,
a = number of levels of factor A = 12,
b = number of levels of factor B = 2,
σ^2 = population variance, and
D^2 = detection threshold

and the MS_{pe} as an estimate of σ^2 yielded a value of

$$\Phi_B = 1.8357 \tag{B-16}$$

To obtain a value of Φ from the ANOVA operating curve, the type I risk α was set to 0.05, the type II risk β was set to 0.10, and the degrees of freedom in the denominator was set to the error degrees of freedom. Then, reading from the operating curve, it was observed that

$$\Phi = 2.4. \tag{B-17}$$

Therefore, a conservative estimate was that the protocol could detect an 1.31 μg/dL increase in resting plasma corticosterone concentrations approximately 90% of the time.

We thank Dr. Russell G. Heikes of Georgia Tech's Department of Industrial and Systems Engineering for his assistance in developing the statistical methodology of this appendix. We also thank Sandra L. Barnes for her assistance in analyzing the corticosterone data set.

APPENDIX C

ACTH SAS FORMATTING PROGRAM

```
1          SAS(R) LOG  CMS SAS 5.16      VM/CMS CMS USER QSECLSB

NOTE: COPYRIGHT (C) 1984,1986 SAS INSTITUTE INC., CARY, N.C.  27511, U.S.A.
NOTE: CMS SAS RELEASE 5.16 AT GEORGIA INSTITUTE OF TECHNOLOGY (03559001).

NOTE: CPUID   VERSION = FF  SERIAL = 012242  MODEL = 4381 .

NOTE: SAS OPTIONS SPECIFIED ARE:
      LEAVE=0

  1 DATA TESTA;
  2 CMS FILEDEF X DISK ACTH DAT A;
  3 CMS FILEDEF 20 DISK ACTH0 LISTING A1 ( BLKSIZE 141 RECFM VBA LRECL 133;
  4 CMS FILEDEF 21 DISK ACTH1 LISTING A1 ( BLKSIZE 141 RECFM VBA LRECL 133;
  5 CMS FILEDEF 22 DISK ACTH2 LISTING A1 ( BLKSIZE 141 RECFM VBA LRECL 133;
  6 CMS FILEDEF 23 DISK ACTH3 LISTING A1 ( BLKSIZE 141 RECFM VBA LRECL 133;
  7 CMS FILEDEF 24 DISK ACTH4 LISTING A1 ( BLKSIZE 141 RECFM VBA LRECL 133;
  8 CMS FILEDEF 25 DISK ACTH5 LISTING A1 ( BLKSIZE 141 RECFM VBA LRECL 133;
  9 CMS FILEDEF 26 DISK ACTH6 LISTING A1 ( BLKSIZE 141 RECFM VBA LRECL 133;
 10 CMS FILEDEF 27 DISK ACTH7 LISTING A1 ( BLKSIZE 141 RECFM VBA LRECL 133;
 11 ARRAY WEEK {33} WKN3 WKN2 MISSN1 WK0-WK24 MISS25 WKP2 MISS27 MISS28 WKP5;
 12 KEEP X XSQR Y Z XZ XSQRZ CASE;
 13 INFILE X;
 14 INPUT CASE 1-3
 15       WKN3 5-7
 16       WKN2 9-11
 17       WK0 13-15
 18       WK1 17-19
 19       WK2 21-23
 20       WK3 25-27
 21       WK4 29-31
 22       WK5 33-35
 23       WK6 37-39
 24       WK7 41-43
 25       WK8 45-47
 26       WK9 49-51
 27       WK10 53-55
 28       WK11 57-59
 29       WK12 61-63
 30       WK13 65-67
 31       WK14 69-71
 32       WK15 73-75
 33       WK16 77-79
 34       WK17 81-83
 35       WK18 85-87
 36       WK19 89-91
 37       WK20 93-95
 38       WK21 97-99
 39       WK22 101-103
 40       WK23 105-107
 41       WK24 109-111
 42       WKP2 113-115
 43       WKP5 117-119
 44 ;
 45 MISSN1=.;
 46 MISS25=.;
 47 MISS27=.;
 48 MISS28=.;
 49 IF CASE < 100 THEN Z = 0;
 50 IF CASE >= 100 THEN Z = 1;
```

```
 51 IF Z=1 THEN CASE=CASE-100;
 52 DO I = 1 TO 33;
 53 X = I-4; XSQR = X*X; XZ = X*Z; XSQRZ = X*X*Z; Y = WEEK {I};OUTPUT;
 54 END;


NOTE: INFILE X IS FILE ACTH DAT A1
NOTE: 151 LINES WERE READ FROM INFILE X.
NOTE: DATA SET WORK.TESTA HAS 4983 OBSERVATIONS AND 7 VARIABLES.
NOTE: THE DATA STATEMENT USED 0.82 SECONDS AND 248K.

 55 PROC CONTENTS;
NOTE: THE PROCEDURE CONTENTS USED 0.22 SECONDS AND 376K AND PRINTED PAGES 1 TO 2.

 56 PROC PRINTTO NEW UNIT=20;

NOTE: THE PROCEDURE PRINTTO USED 0.02 SECONDS AND 312K.

 57 PROC SORT OUT=SCTR;
 58       BY Z X Y;

NOTE: DATA SET WORK.SCTR HAS 4983 OBSERVATIONS AND 7 VARIABLES.
NOTE: THE PROCEDURE SORT USED 1.11 SECONDS AND 6968K.

 59 PROC SUMMARY;
 60       BY Z X;
 61       VAR Y;
 62       OUTPUT OUT=OVLMN MEAN=MEAN;
NOTE: THE DATA SET WORK.OVLMN HAS 66 OBSERVATIONS AND 5 VARIABLES.
NOTE: THE PROCEDURE SUMMARY USED 0.85 SECONDS AND 440K.

 63 DATA SACTH;
 64       SET SCTR OVLMN;
 65       BY Z;

NOTE: DATA SET WORK.SACTH HAS 5049 OBSERVATIONS AND 10 VARIABLES.
NOTE: THE DATA STATEMENT USED 0.83 SECONDS AND 312K.

 66 PROC PLOT NOLEGEND DATA=SACTH;
 67       BY Z;
 68       PLOT MEAN*X='X' Y*X='.' / VAXIS=0 TO 225 BY 25 OVERLAY;
 69       TITLE 'ACTH SCATTER DIAGRAM';
NOTE: THE PROCEDURE PLOT USED 1.58 SECONDS AND 440K AND PRINTED PAGES 3 TO 4.

 70 PROC PRINTTO NEW UNIT=21;

NOTE: THE PROCEDURE PRINTTO USED 0.02 SECONDS AND 312K.

 71 PROC PLOT NOLEGEND DATA=SACTH;
 72       PLOT MEAN*X='X' / VAXIS=0 TO 225 BY 25;
 73       TITLE 'Mean Plasma ACTH Concentration Versus Time';
NOTE: THE PROCEDURE PLOT USED 1.23 SECONDS AND 440K AND PRINTED PAGE 5.

 74 PROC PRINTTO NEW UNIT=22;
 75       TITLE 'ACTH ANALYSIS';

NOTE: THE PROCEDURE PRINTTO USED 0.02 SECONDS AND 312K.
```

```
3          SAS(R) LOG  CMS SAS 5.16      VM/CMS CMS USER QSECLSB

 76 PROC DATASETS;
 77
LIST OF MEMBERS BEFORE UPDATE OF DIRECTORY.
NAME     MEMTYPE             OBS   TRACKS PROT
OVLMN    /DATA                66        1
SACTH    /DATA              5049        1
SCTR     /DATA              4983        1
TESTA    /DATA              4983        1
 77       DELETE SCTR;
 78       DELETE OVLMN;
 79       DELETE TESTA;
LIST OF MEMBERS AFTER UPDATE OF DIRECTORY.
NAME     MEMTYPE             OBS   TRACKS PROT
SACTH    /DATA              5049        1
NOTE: THE PROCEDURE DATASETS USED 0.13 SECONDS AND 440K.

 80 PROC STEPWISE DATA=SACTH;
 81       MODEL Y = X XSQR Z XZ XSQRZ / STEPWISE MAXR;
NOTE: THE PROCEDURE STEPWISE USED 0.82 SECONDS AND 440K AND PRINTED PAGES 6 TO 9.

 82 PROC PRINTTO NEW UNIT=23;

NOTE: THE PROCEDURE PRINTTO USED 0.02 SECONDS AND 312K.

 83 PROC REG;
 84       MODEL Y = X XSQR XZ / PARTIAL;
 85       ID CASE;
NOTE: ACOV AND SPEC OPTION ONLY VALID WITH RAWDATA
NOTE: THE PROCEDURE REG USED 2.83 SECONDS AND 632K AND PRINTED PAGES 10 TO 14.

 86 PROC PRINTTO NEW UNIT=24;

NOTE: THE PROCEDURE PRINTTO USED 0.02 SECONDS AND 312K.

 87 PROC GLM;
 88       CLASS X Z;
 89       MODEL Y = X X*X X*Z;
NOTE: THE PROCEDURE GLM USED 5.17 SECONDS AND 1016K AND PRINTED PAGES 15 TO 16.

 90 PROC PRINTTO NEW UNIT=25;
 91 *-------------------------------------------------------------------*
 92 *                                                                   *
 93 *     to obtain tables listing the variance inflation factors,      *
 94 *     influence statistics, and tolerances, the following SAS       *
 95 *     statements were used in this partition:                       *
 96 *                                                                   *
 97 *     PROC REG;                                                     *
 98 *          MODEL Y = X XSQR XZ / TOL VIF INFLUENCE;                 *
 99 *          ID CASE;                                                 *
100 *          OUTPUT OUT=RACTH P=PREDICT R=RESID STUDENT=STUDENT;      *
101 *                                                                   *
102 *-------------------------------------------------------------------*;

NOTE: THE PROCEDURE PRINTTO USED 0.04 SECONDS AND 312K.

103 PROC REG;
104     MODEL Y = X XSQR XZ / I SS1 SS2 STB COVB CORRB SEQB COLLIN
105                           COLLINOINT ACOV P R CLM;
```

```
106     ID CASE;
107     OUTPUT OUT=RACTH P=PREDICT R=RESID STUDENT=STUDENT;
NOTE: THE DATA SET WORK.RACTH HAS 5049 OBSERVATIONS AND 13 VARIABLES.
NOTE: THE PROCEDURE REG USED 11.26 SECONDS AND 632K AND PRINTED PAGES 17 TO 123.

108 PROC PRINTTO NEW UNIT=26;

NOTE: THE PROCEDURE PRINTTO USED 0.02 SECONDS AND 312K.

109 PROC PLOT DATA=RACTH;
110      PLOT RESID*X='*';
111      PLOT RESID*PREDICT='*' / HAXIS = 80 TO 140 BY 5;
112      PLOT STUDENT*X='*';
113      PLOT STUDENT*PREDICT='*' / HAXIS = 80 TO 140 BY 5;
114      TITLE 'ACTH RESIDUAL PLOTS';
NOTE: THE PROCEDURE PLOT USED 2.06 SECONDS AND 440K AND PRINTED PAGES 124 TO 127.

115 PROC PRINTTO NEW UNIT=27;

NOTE: THE PROCEDURE PRINTTO USED 0.02 SECONDS AND 312K.

116 PROC PLOT DATA=RACTH;
117      BY Z;
118      PLOT RESID*CASE='*' / HAXIS = 0 TO 90 BY 5;
119      PLOT STUDENT*CASE='*' / HAXIS = 0 TO 90 BY 5;
120      TITLE 'ACTH RESIDUAL PLOTS';
NOTE: THE PROCEDURE PLOT USED 1.67 SECONDS AND 440K AND PRINTED PAGES 128 TO 131.
NOTE: SAS USED 6968K MEMORY.

NOTE: SAS INSTITUTE INC.
      SAS CIRCLE
      PO BOX 8000
      CARY, N.C. 27511-8000
```

APPENDIX D

STEPWISE AND MAXIMUM R^2 REGRESSION PROCEDURES USED TO BUILD ACTH MODEL

STEPWISE REGRESSION PROCEDURE FOR DEPENDENT VARIABLE Y

WARNING: 3891 OBSERVATIONS DELETED DUE TO MISSING VALUES.

NOTE: SLENTRY AND SLSTAY HAVE BEEN SET TO .15 FOR THE STEPWISE TECHNIQUE.

STEP 1 VARIABLE XSQR ENTERED R SQUARE = 0.01039482 C(P) = 31.80680300

	DF	SUM OF SQUARES	MEAN SQUARE	F	PROB>F
REGRESSION	1	15547.07455123	15547.07455123	12.14	0.0005
ERROR	1156	1480109.44099281	1280.37148875		
TOTAL	1157	1495656.51554404			

	B VALUE	STD ERROR	TYPE II SS	F	PROB>F
INTERCEPT	93.16450624				
XSQR	0.02328569	0.00668240	15547.07455123	12.14	0.0005

BOUNDS ON CONDITION NUMBER: 1, 1

STEP 2 VARIABLE X ENTERED R SQUARE = 0.03312359 C(P) = 6.57176702

	DF	SUM OF SQUARES	MEAN SQUARE	F	PROB>F
REGRESSION	2	49541.51089734	24770.75544867	19.78	0.0001
ERROR	1155	1446115.00464670	1252.04762307		
TOTAL	1157	1495656.51554404			

	B VALUE	STD ERROR	TYPE II SS	F	PROB>F
INTERCEPT	99.50985764				
X	-2.14343953	0.41135591	33994.43634611	27.15	0.0001
XSQR	0.11158057	0.01818792	47122.90682396	37.64	0.0001

BOUNDS ON CONDITION NUMBER: 7.575575, 30.3023

STEP 3 VARIABLE XZ ENTERED R SQUARE = 0.03770867 C(P) = 3.07763639

	DF	SUM OF SQUARES	MEAN SQUARE	F	PROB>F
REGRESSION	3	56399.21704805	18799.73901602	15.07	0.0001
ERROR	1154	1439257.29849599	1247.19003336		
TOTAL	1157	1495656.51554404			

	B VALUE	STD ERROR	TYPE II SS	F	PROB>F
INTERCEPT	99.50218899				
X	-1.79395756	0.43677223	21040.05756260	16.87	0.0001
XSQR	0.12385386	0.01889212	53603.15326343	42.98	0.0001
XZ	-0.68515788	0.29219147	6857.70615071	5.50	0.0192

BOUNDS ON CONDITION NUMBER: 8.573899, 62.65483

NO OTHER VARIABLES MET THE 0.1500 SIGNIFICANCE LEVEL FOR ENTRY INTO THE MODEL.

SUMMARY OF STEPWISE REGRESSION PROCEDURE FOR DEPENDENT VARIABLE Y

STEP	VARIABLE ENTERED	VARIABLE REMOVED	NUMBER IN	PARTIAL R**2	MODEL R**2	C(P)	F	PROB>F
1	XSQR		1	0.0104	0.0104	31.8068	12.1426	0.0005
2	X		2	0.0227	0.0331	6.5718	27.1511	0.0001
3	XZ		3	0.0046	0.0377	3.0776	5.4985	0.0192

MAXIMUM R-SQUARE IMPROVEMENT FOR DEPENDENT VARIABLE Y

WARNING: 3891 OBSERVATIONS DELETED DUE TO MISSING VALUES.

STEP 1 VARIABLE XSQR ENTERED R SQUARE = 0.01039482 C(P) = 31.80680300

	DF	SUM OF SQUARES	MEAN SQUARE	F	PROB>F
REGRESSION	1	15547.07455123	15547.07455123	12.14	0.0005
ERROR	1156	1480109.44099281	1280.37148875		
TOTAL	1157	1495656.51554404			

	B VALUE	STD ERROR	TYPE II SS	F	PROB>F
INTERCEPT	93.16450624				
XSQR	0.02328569	0.00668240	15547.07455123	12.14	0.0005

BOUNDS ON CONDITION NUMBER: 1, 1

THE ABOVE MODEL IS THE BEST 1 VARIABLE MODEL FOUND.

STEP 2 VARIABLE X ENTERED R SQUARE = 0.03312359 C(P) = 6.57176702

	DF	SUM OF SQUARES	MEAN SQUARE	F	PROB>F
REGRESSION	2	49541.51089734	24770.75544867	19.78	0.0001
ERROR	1155	1446115.00464670	1252.04762307		
TOTAL	1157	1495656.51554404			

	B VALUE	STD ERROR	TYPE II SS	F	PROB>F
INTERCEPT	99.50985764				
X	-2.14343953	0.41135591	33994.43634611	27.15	0.0001
XSQR	0.11158057	0.01818792	47122.90682396	37.64	0.0001

BOUNDS ON CONDITION NUMBER: 7.575575, 30.3023

THE ABOVE MODEL IS THE BEST 2 VARIABLE MODEL FOUND.

STEP 3 VARIABLE XZ ENTERED R SQUARE = 0.03770867 C(P) = 3.07763639

	DF	SUM OF SQUARES	MEAN SQUARE	F	PROB>F
REGRESSION	3	56399.21704805	18799.73901602	15.07	0.0001
ERROR	1154	1439257.29849599	1247.19003336		
TOTAL	1157	1495656.51554404			

	B VALUE	STD ERROR	TYPE II SS	F	PROB>F
INTERCEPT	99.50218899				
X	-1.79395756	0.43677223	21040.05756260	16.87	0.0001
XSQR	0.12385386	0.01889212	53603.15326343	42.98	0.0001
XZ	-0.68515788	0.29219147	6857.70615071	5.50	0.0192

BOUNDS ON CONDITION NUMBER: 8.573899, 62.65483

MAXIMUM R-SQUARE IMPROVEMENT FOR DEPENDENT VARIABLE Y

THE ABOVE MODEL IS THE BEST 3 VARIABLE MODEL FOUND.

STEP 4 VARIABLE XSQRZ ENTERED R SQUARE = 0.03850892 C(P) = 4.11872680

	DF	SUM OF SQUARES	MEAN SQUARE	F	PROB>F
REGRESSION	4	57596.11618460	14399.02904615	11.54	0.0001
ERROR	1153	1438060.39935944	1247.23365079		
TOTAL	1157	1495656.51554404			

	B VALUE	STD ERROR	TYPE II SS	F	PROB>F
INTERCEPT	99.14919817				
X	-1.08283623	0.84719341	2037.54983369	1.63	0.2015
XSQR	0.07088105	0.05728047	1909.83153498	1.53	0.2162
XZ	-1.43380998	0.81818658	3830.24287046	3.07	0.0800
XSQRZ	0.05626005	0.05743085	1196.89913655	0.96	0.3275

BOUNDS ON CONDITION NUMBER: 75.42855, 860.8978

THE ABOVE MODEL IS THE BEST 4 VARIABLE MODEL FOUND.

STEP 5 VARIABLE Z ENTERED R SQUARE = 0.03860800 C(P) = 6.00000000

	DF	SUM OF SQUARES	MEAN SQUARE	F	PROB>F
REGRESSION	5	57744.30951763	11548.86190353	9.25	0.0001
ERROR	1152	1437912.20602641	1248.18767884		
TOTAL	1157	1495656.51554404			

	B VALUE	STD ERROR	TYPE II SS	F	PROB>F
INTERCEPT	97.99515863				
X	-0.79853500	1.18282235	568.89059651	0.46	0.4997
XSQR	0.05749827	0.06922470	861.12704877	0.69	0.4064
Z	1.47211264	4.27234832	148.19333303	0.12	0.7305
XZ	-1.76801846	1.26914033	2422.33491213	1.94	0.1639
XSQRZ	0.07120070	0.07197893	1221.34298953	0.98	0.3228

BOUNDS ON CONDITION NUMBER: 118.2663, 1860.332

THE ABOVE MODEL IS THE BEST 5 VARIABLE MODEL FOUND.

61/62

APPENDIX E

ACTH LACK-OF-FIT TEST

ACTH ANALYSIS 15:36 TUESDAY, JULY 7, 1987

GENERAL LINEAR MODELS PROCEDURE

DEPENDENT VARIABLE: Y

SOURCE	DF	SUM OF SQUARES	MEAN SQUARE	F VALUE	PR > F	R-SQUARE
MODEL	45	236520.37628796	5256.00836195	4.64	0.0001	0.158138
ERROR	1112	1259136.13925609	1132.31669199		ROOT MSE	
CORRECTED TOTAL	1157	1495656.51554404			33.64991340	

SOURCE	DF	TYPE I SS	F VALUE	PR > F	DF	TYPE III SS	F VALUE
X	28	194947.14800659	6.15	0.0001	28	166337.49533754	5.25
X*Z	17	41573.22828136	2.16	0.0041	17	41573.22828136	2.16

this term is solely a measure of sum-of-squares pure error.

Partitioning SS_E into SS_{pe} and SS_{lof}

SS_E = 1439257.30 df = 1154

SS_{pe} = 1259136.14 df = 1112

SS_{lof} = 180121.16 df = 42

MS_{lof} = 4288.5990

MS_{pe} = 1132.3167

$$F_o = \frac{MS_{lof}}{MS_{pe}} = 3.7875$$

$F_{0.05,\ 42,\ 1112} \sim 1.43$

ACTH ANALYSIS

ANALYSIS OF VARIANCE

SOURCE	DF	SUM OF SQUARES	MEAN SQUARE	F VALUE	PROB>F
MODEL	3	56399.21705	18799.73902	15.074	0.0001
ERROR	1153	1439257.30	1247.98902		
C TOTAL	1157	1495656.52			

ROOT MSE	35.31558	R-SQUARE	0.0377
DEP MEAN	95.41192	ADJ R-SQ	0.0352
C.V.	37.0138		

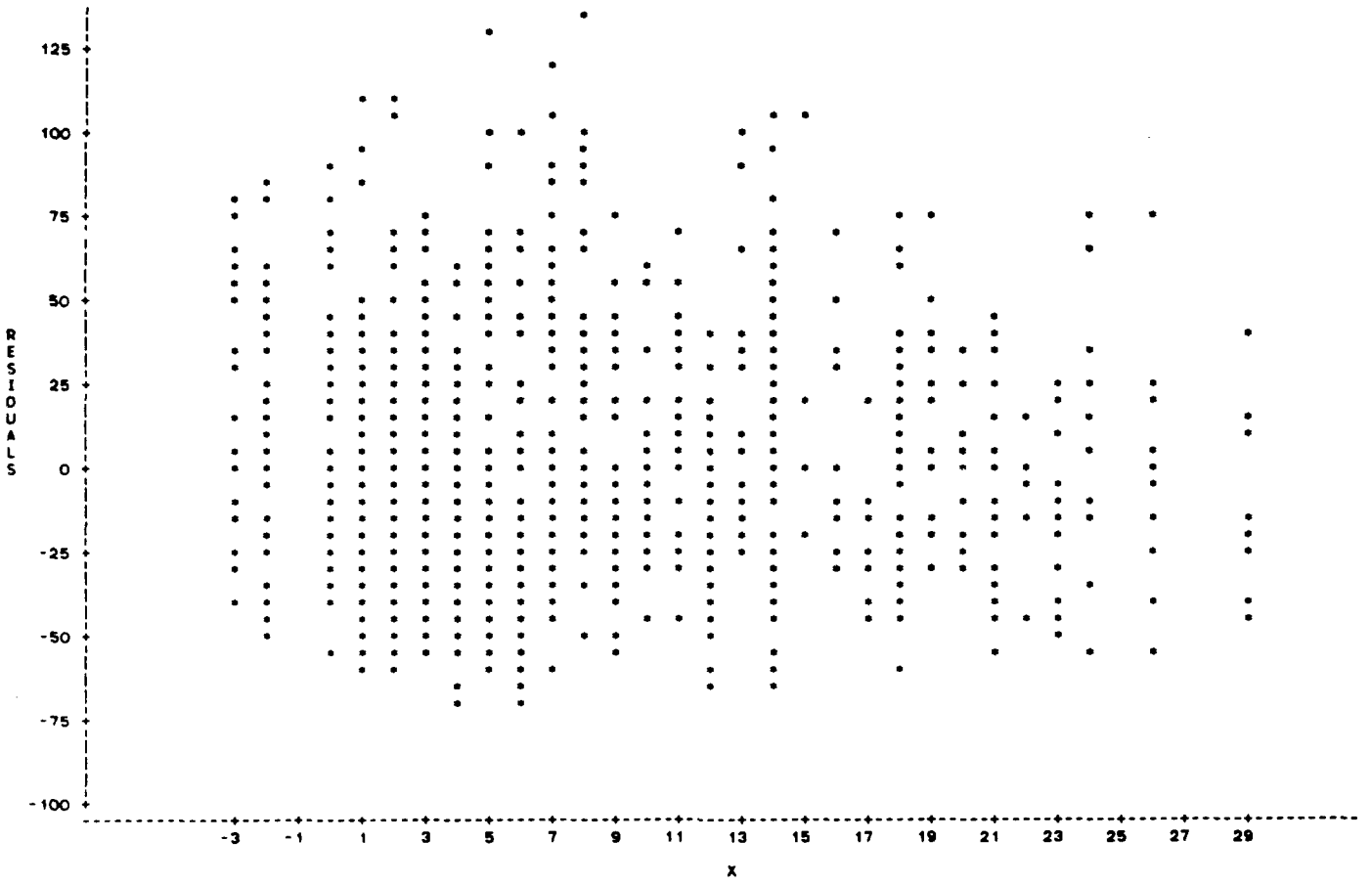
PARAMETER ESTIMATES

VARIABLE	DF	PARAMETER ESTIMATE	STANDARD ERROR	T FOR HO: PARAMETER=0	PROB > |T|
INTERCEP	1	99.50218899	1.72029112	57.840	0.0001
X	1	-1.79395756	0.43677223	-4.107	0.0001
XSQR	1	0.12385386	0.01889212	6.556	0.0001
XZ	1	-0.68515788	0.29219147	-2.345	0.0192

this term contains both sum-of-squares pure error and sum-of-squares lack-of-fit.

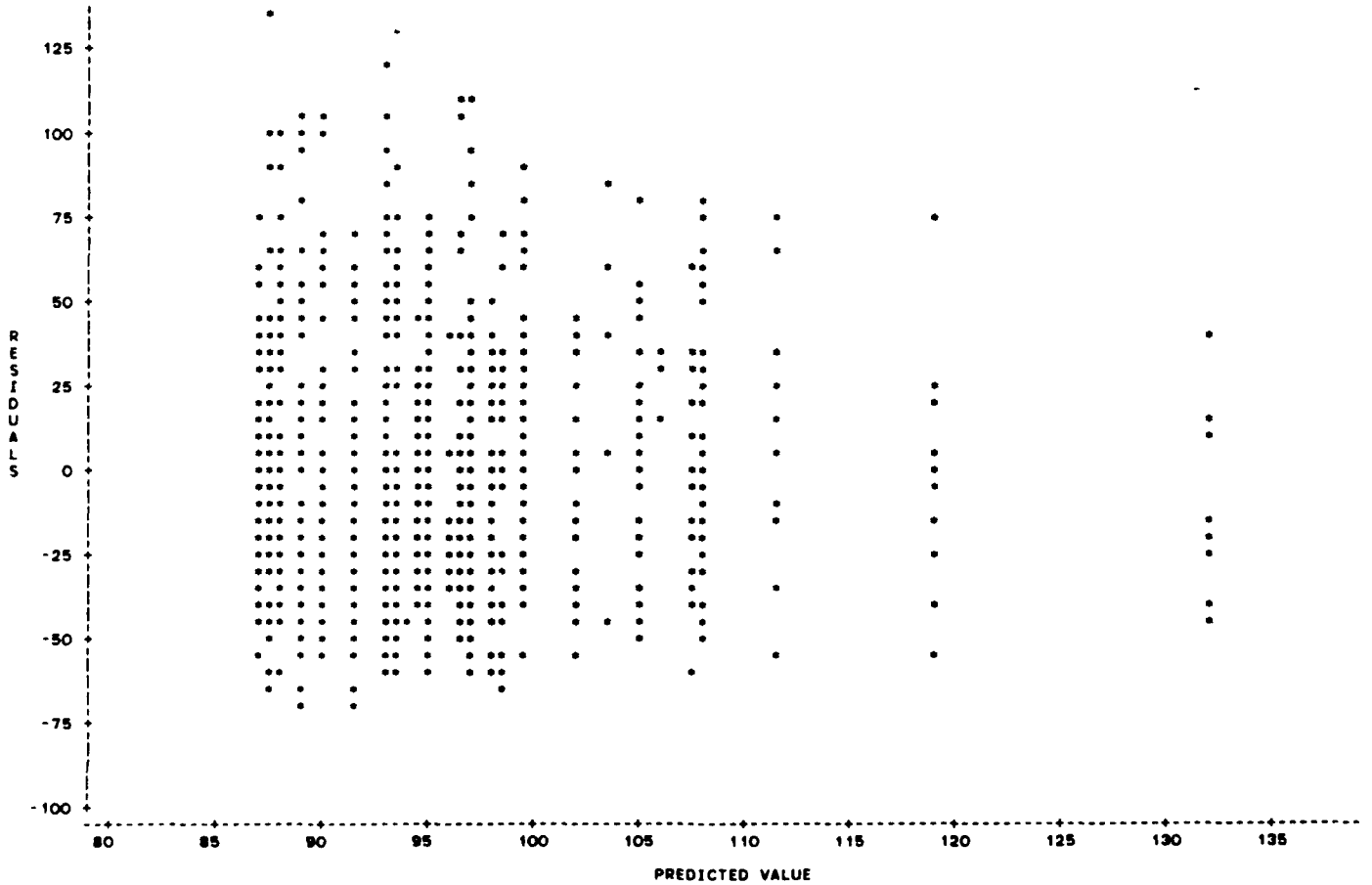
APPENDIX F

ACTH RESIDUAL PLOTS



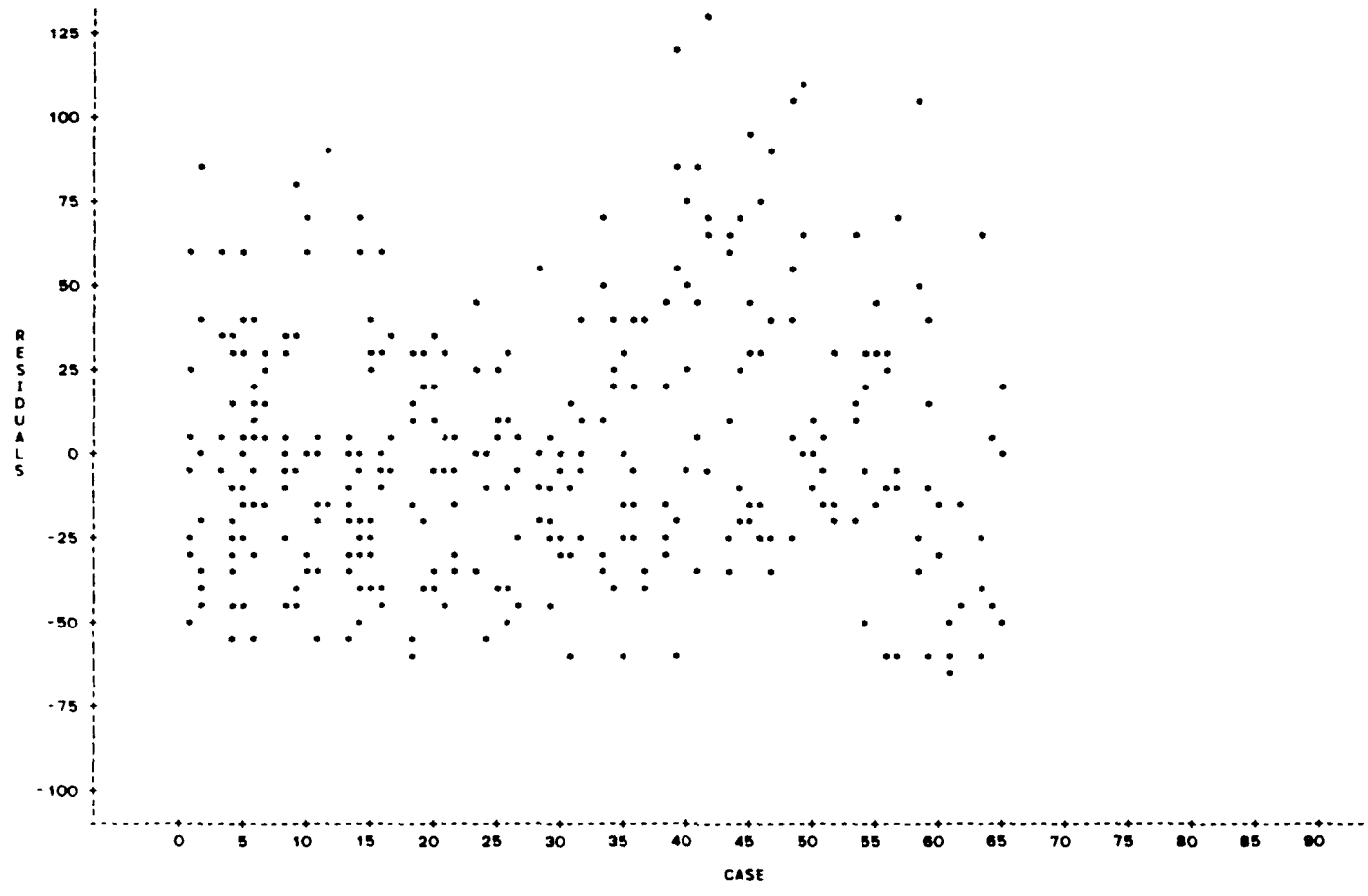
NOTE: 3891 OBS HAD MISSING VALUES 663 OBS HIDDEN

Residuals versus time.



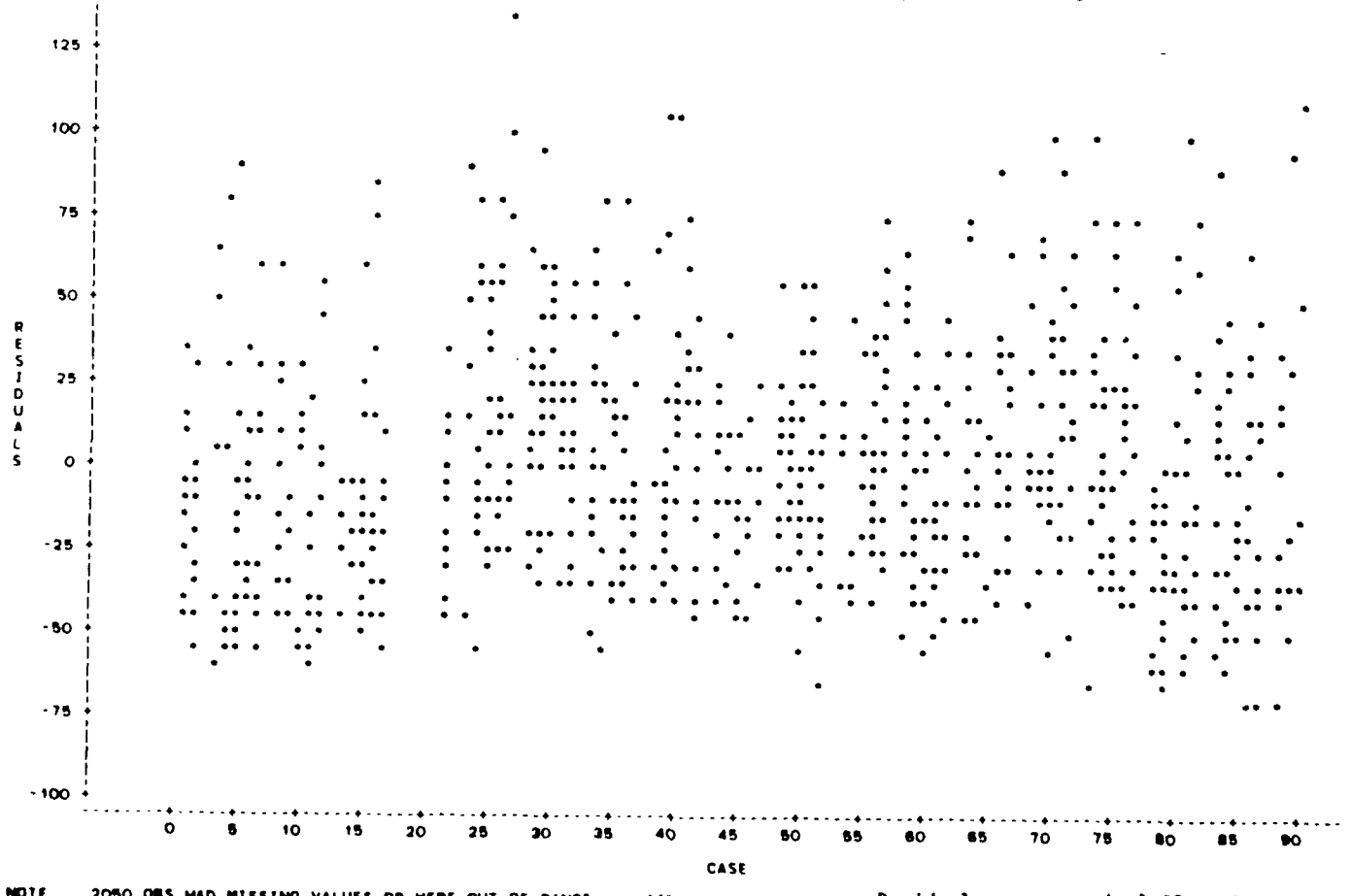
NOTE: 3891 OBS HAD MISSING VALUES OR WERE OUT OF RANGE 690 OBS HIDDEN

Residuals versus predicted value of plasma ACTH concentration.



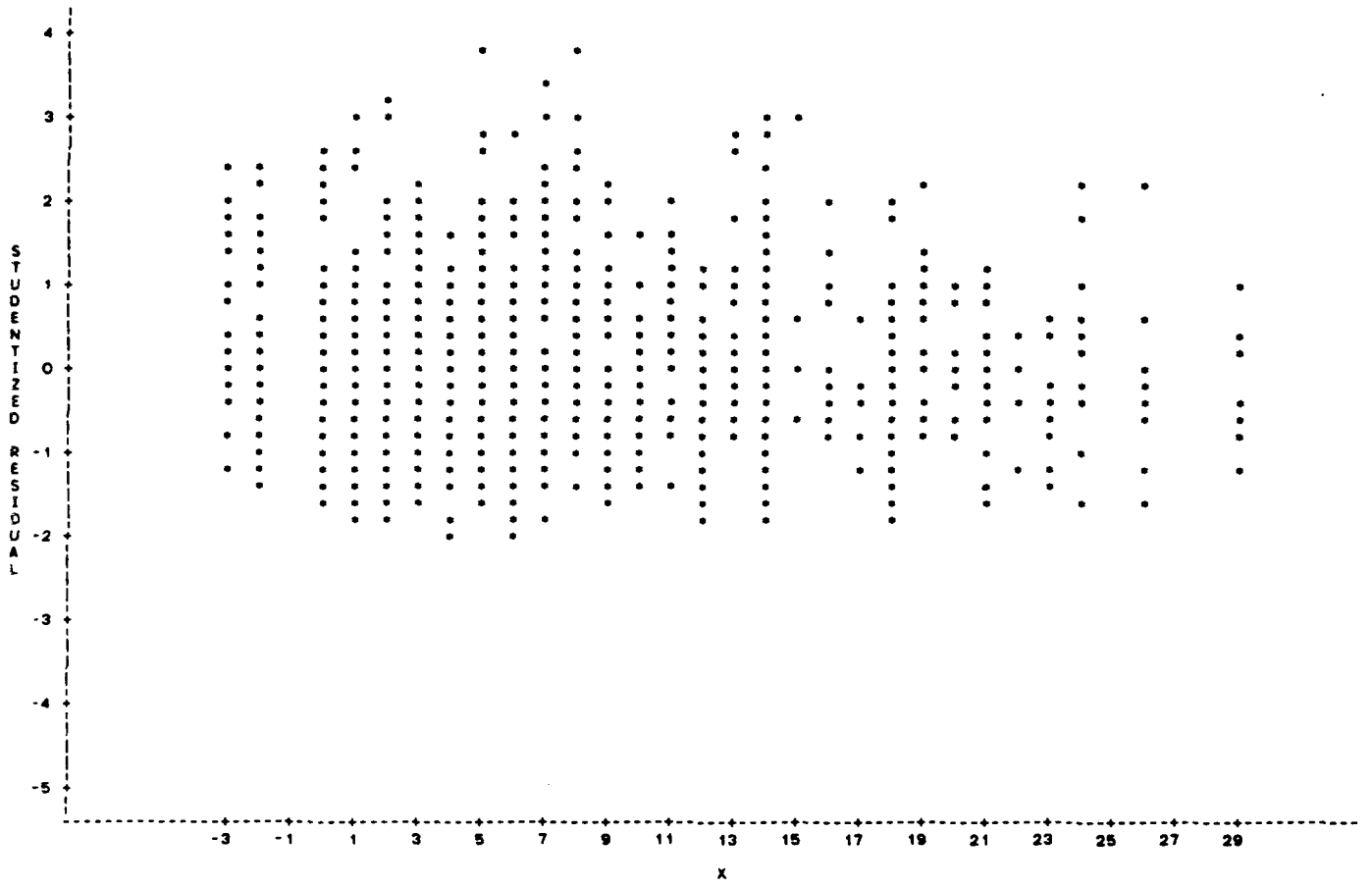
NOTE: 1841 OBS HAD MISSING VALUES OR WERE OUT OF RANGE 33 OBS HIDDEN

Residuals versus animal ID number (sham-exposure group).



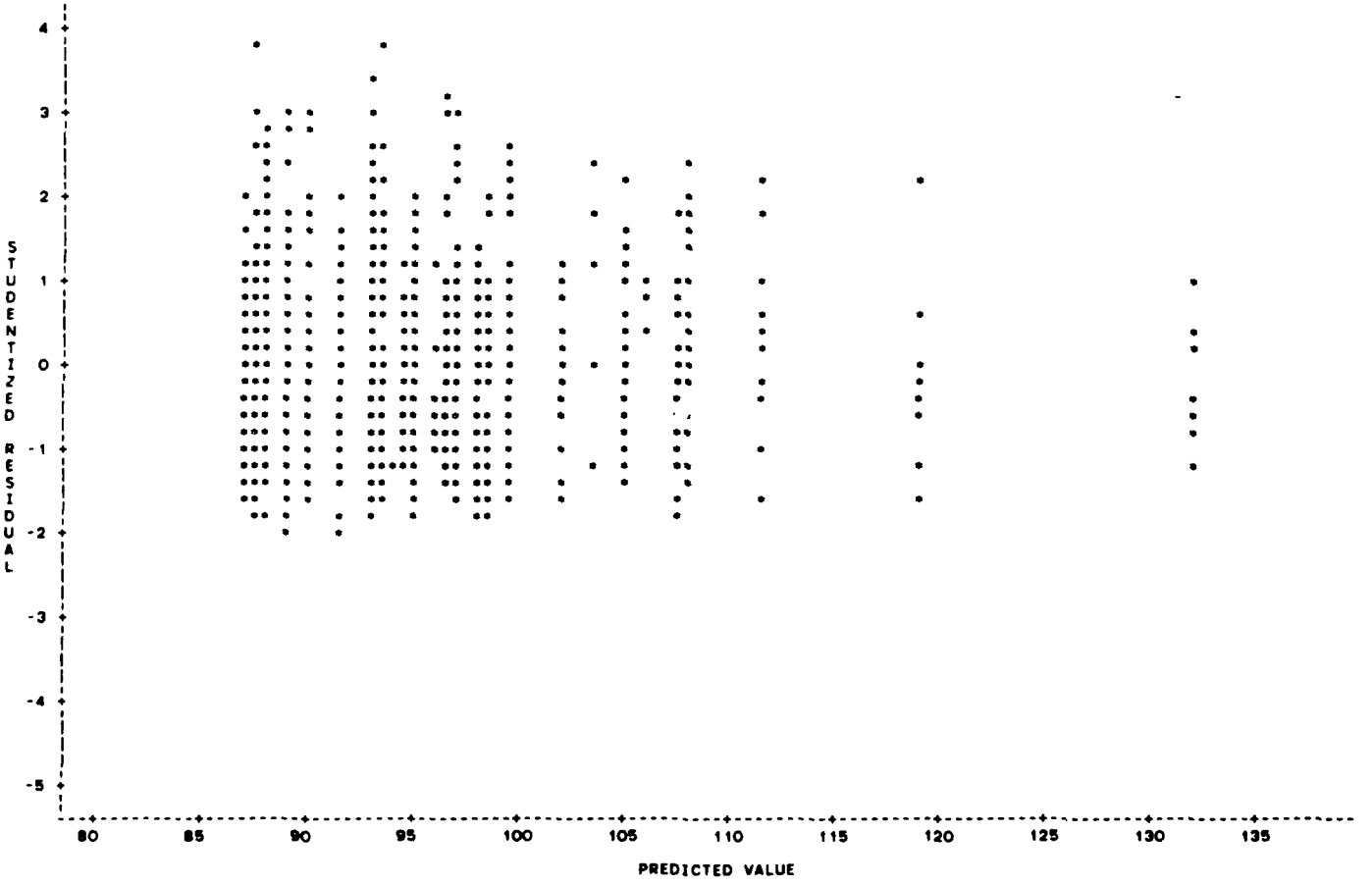
NOTE 2050 OBS HAD MISSING VALUES OR WERE OUT OF RANGE 142 OBS HIDDEN

Residuals versus animal ID number (exposure group).



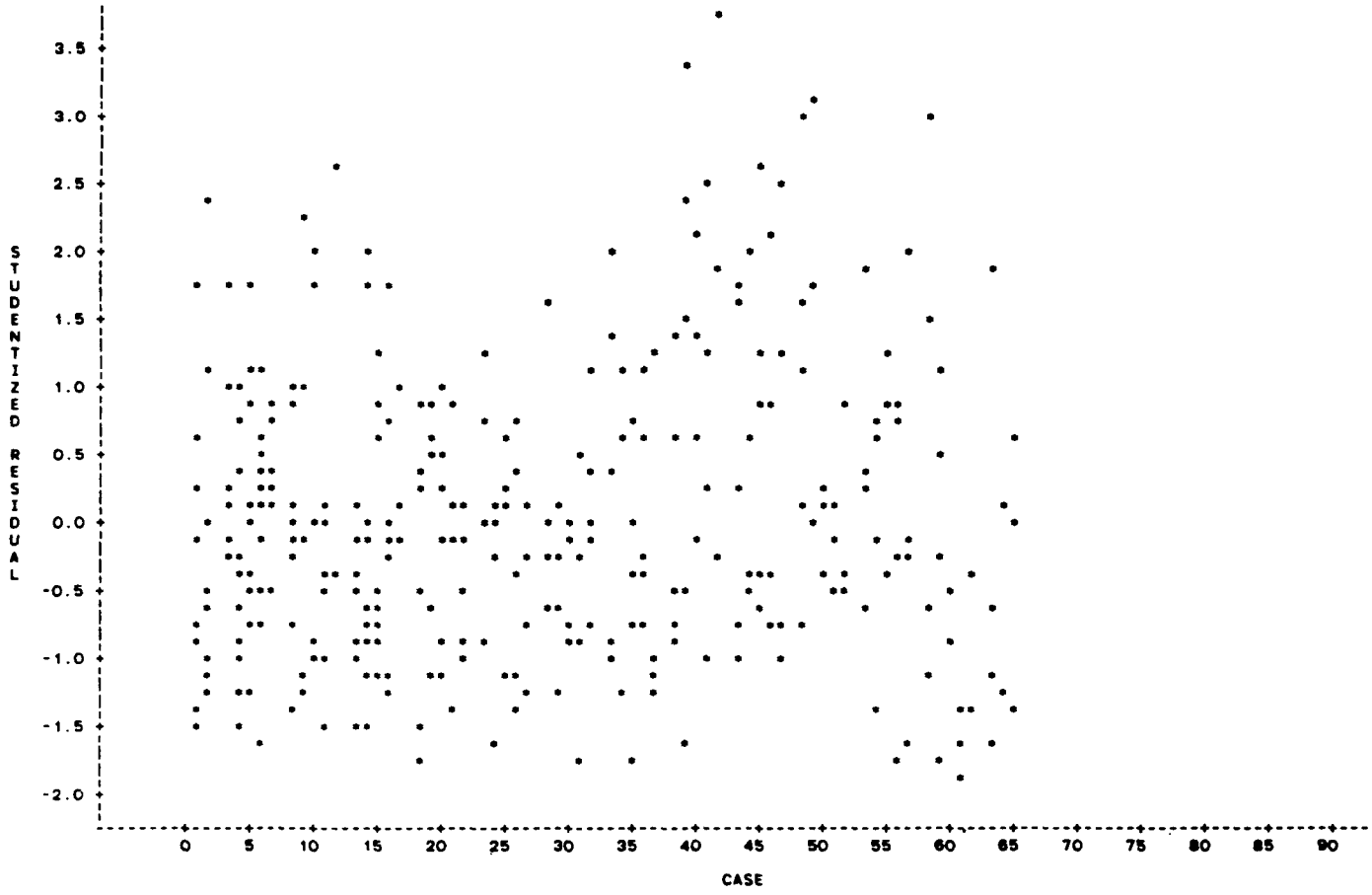
NOTE: 3891 OBS HAD MISSING VALUES 746 OBS HIDDEN

Studentized residuals versus time.



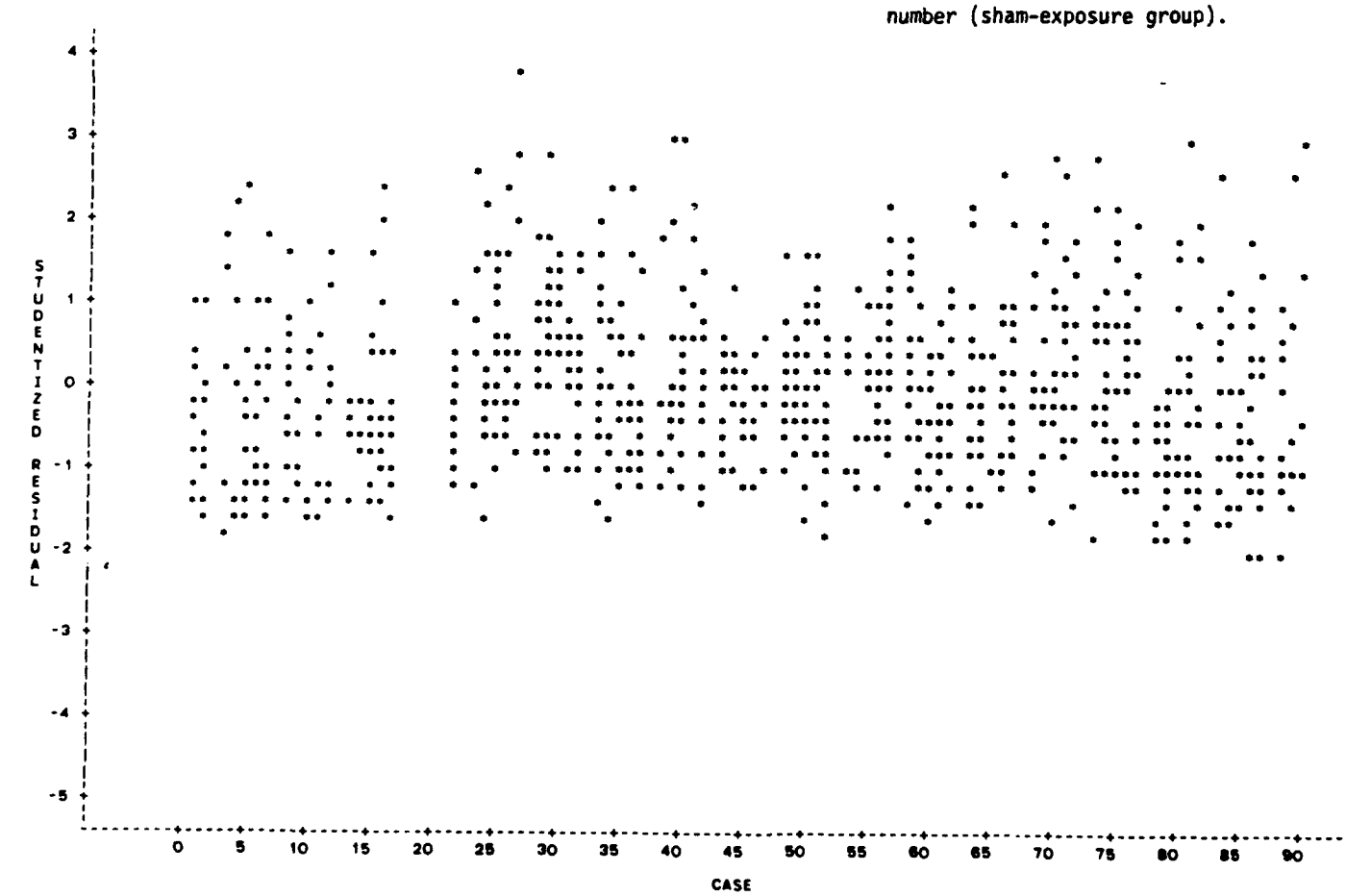
NOTE: 3891 OBS HAD MISSING VALUES OR WERE OUT OF RANGE 771 OBS HIDDEN

Studentized residuals versus predicted value of plasma ACTH concentration.



NOTE: 1841 OBS HAD MISSING VALUES OR WERE OUT OF RANGE 27 OBS HIDDEN

Studentized residuals versus animal ID number (sham-exposure group).



NOTE: 2050 OBS HAD MISSING VALUES OR WERE OUT OF RANGE 176 OBS HIDDEN

Studentized residuals versus animal ID number (exposure group).

APPENDIX G

RAW CORTICOSTERONE DATA SPREADSHEETS

Corticosterone

Control I

Errors

Rat #	Group	TIME -3WK	-2WK	0WK	1WK	2WK	3WK	4WK	5WK	6WK	7WK	8WK	9WK	10WK	11WK	12WK	13WK	14WK	15WK	16WK	17WK	18WK	19WK	20WK	21WK	22WK	23WK	24WK	+2	+5
1					1	7	8	3		4		5	6			21		7				7								
2		20	27	15	2	28	19	7		4		5	2			4		18				8								
3		17	21	12	22	20	20	5																						
4		14	7	7	2	16	11	9		3		8	7			8		5				12								
5		22	12	24	12	28	9	8		4		3	7	10		13														
6		20	20	14	22	39	23	5		4		14	3			6		22				11								
7		14		27	26	14	16	5		4																				
8		8		12	10	16	12	5				15				5		5				12								
9		16		16		3	8						21					16				19								
10		21		18		9	7				17		9					19				21								
11		27		9		7					14																			
12		17		27		1	6	2	2		8		30					18				17								
13		30		14		6	9	8	2		24		11					19				12								

1
69

Corticost.

Control II

Rat #	Group	TIME -3WK	-2WK	0WK	1WK	2WK	3WK	4WK	5WK	6WK	7WK	8WK	9WK	10WK	11WK	12WK	13WK	14WK	15WK	16WK	17WK	18WK	19WK	20WK	21WK	22WK	23WK	24WK	+2	+5
14		21		23		8	6	8	3		7		13					38												
15		19		6	3	12		11		19								4												
16		14		10	10	11		38		9					26			27												
17		27		19	5	2		15		19					16			23												
18		12		10	2	6		21		6								1												
19					12	9		6		27					30			22												
20					4	3		35							26			8												
21					1	3				20					9			27												
22					6	7		24		24					19			7												
23					5	2		48		7					11			19												
24					1	12		7		18								10												
25					34	2		14		24					18			11												
26					1	11		13		10					10			18												

9

Corticost.
Control III

Rat #	Group	-3WK	-2WK	0WK	TIME 1WK	2WK	3WK	4WK	5WK	6WK	7WK	8WK	9WK	10WK	11WK	12WK	13WK	14WK	15WK	16WK	17WK	18WK	19WK	20WK	21WK	22WK	23WK	24WK	+2	+5
27					10	3		28		12					12															
28					13	4				6					11															
29					11	12		29		17					11															
30					24	6		8		4					19															
31					7	21									25															
32					1	12		7		6					7															
33					22	3		6		8					7															
34					23	3		18		17					16															
35					19	12		7		2					17				26											
36						9			8	34	28	16			19				31											
37						12			20	15	35																			
38						12			3	13	40	71		16					20											
39						12			14		28	29							9											

Corticost.
Control IV

Rat #	Group	-3WK	-2WK	0WK	TIME 1WK	2WK	3WK	4WK	5WK	6WK	7WK	8WK	9WK	10WK	11WK	12WK	13WK	14WK	15WK	16WK	17WK	18WK	19WK	20WK	21WK	22WK	23WK	24WK	+2	+5
40						6			31	13	40	21		16																
41						9			9	53	28	29																		
42						25			21	27	27	18																		
43						12			21	12	14	20																		
44						9			32	26	38	33																		
45						6			5	26	23	22		22																
46									6	18	29																			
47						15			19	19	40	18																		
48						12			23	24	22	31		29																
49						32			4	32																				
50						13			14			12					18					24								
51						9			23			7					21					19								
52						29			22			17					16					29								

Caticost.

V Control ♀V Control V

Rec #	Group	-3WK	-2WK	0WK	TIME 1WK	2WK	3WK	4WK	5WK	6WK	7WK	8WK	9WK	10WK	11WK	12WK	13WK	14WK	15WK	16WK	17WK	18WK	19WK	20WK	21WK	22WK	23WK	24WK	+2	+5
53						16			6									27				22									
54						49			6									21				19									
55						57			30									19				27									
56						17			20									23				8									
57						16			21									8				16									
58						9			12									14				8									
59						10			13									20				4									
60						2			15																					
61									9									5				9									
62						10			9																					
63						35			5									5				6									
64						30			8																					
65						28			2									18				12						.			

Caticostera

M - I

Rec #	Group	-3WK	-2WK	0WK	TIME 1WK	2WK	3WK	4WK	5WK	6WK	7WK	8WK	9WK	10WK	11WK	12WK	13WK	14WK	15WK	16WK	17WK	18WK	19WK	20WK	21WK	22WK	23WK	24WK	+2	+5
1	♂	1	33	16		7	2		13	1	9	12	←	←															14	12
2		2	15	8		23	5	3	3	2	9	14																		
3		22	21	14		3	3																							
4		17	25	12		4	1	9																						
5		12	5	7		6	17	6	5	1	7	18	←	←															8	18
6		21	3			24	3	4	3	1		20																		
7		25	32	26	14	16			1	1		22																		
8		10	14	10	9	2			2	4	1	17	←	←															23	7
9		11	22	7			11		5																					
10		17	25	28	2																									
11		12	5	20	14		12		10	1																				
12			4	15	16		2	2	7	1		19	←	←															17	19
13	♂♂	8	17	27		2					1																			

Corticost

M - II

Rat #	Group	-3WK	-2WK	0WK	TIME 1WK	2WK	3WK	4WK	5WK	6WK	7WK	8WK	9WK	10WK	11WK	12WK	13WK	14WK	15WK	16WK	17WK	18WK	19WK	20WK	21WK	22WK	23WK	24WK	+2	+5
14		19	13	12		9																								
15		21	26	9		8	6	4	10	4	4	14		5				12				7	←	←					10	11
16		20	25	17					13	10	12																			
17		21	24	9		1	3	9	14	4	3	19		9				13				7	←	←					7	17
18		17	22	11					8	11	13																			
19		18	7	10			3	10																						
20		11	16	11		10	5	4	8	4	9	5		8				4												
21			15						4	10																				
22	B	18	9	12	3	[illegible]	58	8	7			4		1		1		20				4	←	←					14	9
23		22	14	21	2	37	17	5	1		3		2		9		17													
24		19	29	31	40	29		17	1		3		9		1															
25		41	19	17	13	35	9	9		6	10	34	16	21	24															
26			29	30	17	31	20	10	9	5		11		13		13		1												

Corticosterone

M - III

Rat #	Group	-3WK	-2WK	0WK	TIME 1WK	2WK	3WK	4WK	5WK	6WK	7WK	8WK	9WK	10WK	11WK	12WK	13WK	14WK	15WK	16WK	17WK	18WK	19WK	20WK	21WK	22WK	23WK	24WK	+2	+5
27		24	17	17	29	32	21		18		1		4																	
28		30	27	20	33	27				15		3		15				15				12	←	←					17	9
29		24	22	18	20	14		3		21		13				1		11				13	←	←					6	6
30			11	12	25	16		4		18		13				5		8				7								
31			26	6	20	2	16	12																						
32			8	22	18	32		1		1		4		11		8		2		2		12	17	[illegible]						
33			2	14	15	12	17	4				1		16		18		4				5	←	←					19	21
34			23	4	10		17	13			13		16					37				9	←	←					9	12
35		29	24	5	1		13	1			11		12		11	1		3				19								
36			19	12	4		2	1	20		2		17		21		1		1			19								
37			12	9	5		13		2		2		1		1		10		1											
38			8	7	4		3				3		7		4		1		12			6								
39			12	13	10		2	18	1		1		12					36				14	←	←					15	14

Corticosterone

M-IV

Rat #	Group	-3WK	-2WK	0WK	TIME 1WK	2WK	3WK	4WK	5WK	6WK	7WK	8WK	9WK	10WK	11WK	12WK	13WK	14WK	15WK	16WK	17WK	18WK	19WK	20WK	21WK	22WK	23WK	24WK	+2	+5	
40			17	7	5		3	2	2		1		13						26			19	7	←	←				10	15	D
41			16	18	1		16				9																				
42			7	14	4		1	12	2		1		7	5		11		37		16		3									310
43			14	21	7		12				12		38						3			7		←	←				12	7	
44	F		12	15	1	2		1		28																					F
45			8	7	6	7		1		14																					
46			16	7	3	4		15		16					21				5			5		←	←				9	9	
47			10	10	3	3		10																							
48			15	12	17	1		1		20		8		1	8		1		6			14		←	←				18	7	318
49			12	14	7	16		10		7		7		2	1		2		9												
50			27	13	9	7		1		15		2	9		1		34		8	20	5	15		←	←				27	3	
51					11	4		2		19		11			3																
52			19	22	9	23		1		13		32		1		1	1		21		20	3		←	←				20	14	319

Corticosterone

M-V

Rat #	Group	-3WK	-2WK	0WK	TIME 1WK	2WK	3WK	4WK	5WK	6WK	7WK	8WK	9WK	10WK	11WK	12WK	13WK	14WK	15WK	16WK	17WK	18WK	19WK	20WK	21WK	22WK	23WK	24WK	+2	+5	
53					2	9		1		15																					
54					12	10		1		11																					
55					6	1		10		35					12																
56		19	14	10	10	1		3		10					10					7					11	16	15		14	17	
57			27	21	8	11		4		17					13					12					25	20	13		22	19	
58		19	16	20	8	1				22					10					14					21	15			21	24	
59			32	26	16	1		17		23					25					19					17				18	13	↑
60		22	8	17	19	1		21		8					12					22			4		29	22	24		24	20	
61		27	19	22	12	1		3		11					16								7		11	24	21		19	18	
62			7	16	1	13		2		18					18					10			16		26	15	22		6	14	
63		22	17	15	1	1		23		3					17								18		11	27	21		18	17	
64			7	16	1	1		6		22					14					7					11	24	21		24		↓
65	M	20	8	16	8	14		4															18		21	21	14		13	15	

Corticosterone

M-VI

Rat #	Group	-3WK	-2WK	0WK	1WK	2WK	3WK	4WK	5WK	6WK	7WK	8WK	9WK	10WK	11WK	12WK	13WK	14WK	15WK	16WK	17WK	18WK	19WK	20WK	21WK	22WK	23WK	24WK	+2	+5
65			11	17	8	17					8	17				22	25	14			18	24	22	←	←				19	16
67			26	21	12	20					12	20				9	16	8			10	13	28	←	←				27	13
68			22	27	35	11					35	12				27	24					16								
69					8	14					8	15				6	12	23			36	26								
70					6	12					6	16				8	9	18			23	15								
71			14	14	15	17					12	12				15	26	16			11		27	←	←				20	16
72					12	5					10	6				19	11				27	17								
73					29	20					29	24				9	27	7			12	12		←	←				18	14
74					19	8					18	10				11	29	26				16								
75					24	12					20	17				22	21	18			17									
76			26	25	22	11					22	13					15				25	25	20							
77					37	11					37	14					17	24			3	20								
78			17	14	1	4	14	1	5	29	13	7	7	44		1		30		2		7	7							

Corticosterone

M-VII

Rat #	Group	-3WK	-2WK	0WK	1WK	2WK	3WK	4WK	5WK	6WK	7WK	8WK	9WK	10WK	11WK	12WK	13WK	14WK	15WK	16WK	17WK	18WK	19WK	20WK	21WK	22WK	23WK	24WK	+2	+5
79			8	10	2	10	3	5	4	16	3	14	20	14		1		19		2		·								
80			1	12	1	1	4	2	15		6	30	12	20		17		3		7		4								
81			16	7	13	3	2	16	1	17	10	22	19	9		1		4		4		15	34							
82			12	13	1	1	3	6	17		3	8	13	17		2		1		14		8	12							
83			21	19		16	4	1			12	20	25	12		1		3		16		1	16							
84			19	25	5	2	10	2	1	24	8	18	5	2		1		4		2		4	27							
85			18	7	10	11	3	6	3	36	20	21		12		9		3		1		1								
86			13	18	27	1	2	4	6		14	19	9	23		1		3		2		9	17							
87			41	40	42	2	2	6	7	17	15	28	16	17		1		3		9		1	17							
88																														
89																														
90																														
91																														

APPENDIX H

CORTICOSTERONE SAS FORMATTING PROGRAM

```
1          SAS(R) LOG  CMS SAS 5.16      VM/CMS CMS USER QSECLSB

NOTE: COPYRIGHT (C) 1984,1986 SAS INSTITUTE INC., CARY, N.C.  27511, U.S.A.
NOTE: CMS SAS RELEASE 5.16 AT GEORGIA INSTITUTE OF TECHNOLOGY (03559001).

NOTE: CPUID   VERSION = FF  SERIAL = 012242  MODEL = 4381 .

NOTE: SAS OPTIONS SPECIFIED ARE:
      LEAVE=0

  1 DATA TESTC;
  2 CMS FILEDEF X DISK CORTICO DAT A;
  3 CMS FILEDEF 20 DISK CORTICO0 LISTING A1 ( BLKSIZE 141 RECFM VBA LRECL 133;
  4 CMS FILEDEF 21 DISK CORTICO1 LISTING A1 ( BLKSIZE 141 RECFM VBA LRECL 133;
  5 CMS FILEDEF 22 DISK CORTICO2 LISTING A1 ( BLKSIZE 141 RECFM VBA LRECL 133;
  6 CMS FILEDEF 23 DISK CORTICO3 LISTING A1 ( BLKSIZE 141 RECFM VBA LRECL 133;
  7 CMS FILEDEF 24 DISK CORTICO4 LISTING A1 ( BLKSIZE 141 RECFM VBA LRECL 133;
  8 CMS FILEDEF 25 DISK CORTICO5 LISTING A1 ( BLKSIZE 141 RECFM VBA LRECL 133;
  9 CMS FILEDEF 26 DISK CORTICO6 LISTING A1 ( BLKSIZE 141 RECFM VBA LRECL 133;
 10 CMS FILEDEF 27 DISK CORTICO7 LISTING A1 ( BLKSIZE 141 RECFM VBA LRECL 133;
 11 ARRAY WEEK {33} WKN3 WKN2 MISSN1 WK0-WK24 MISS25 WKP2 MISS27 MISS28 WKP5;
 12 KEEP X XSQR Y Z XZ XSQRZ CASE;
 13 INFILE X;
 14 INPUT CASE 1-3
 15       WKN3 5-6
 16       WKN2 8-9
 17       WK0 11-12
 18       WK1 14-15
 19       WK2 17-18
 20       WK3 20-21
 21       WK4 23-24
 22       WK5 26-27
 23       WK6 29-30
 24       WK7 32-33
 25       WK8 35-36
 26       WK9 38-39
 27       WK10 41-42
 28       WK11 44-45
 29       WK12 47-48
 30       WK13 50-51
 31       WK14 53-54
 32       WK15 56-57
 33       WK16 59-60
 34       WK17 62-63
 35       WK18 65-66
 36       WK19 68-69
 37       WK20 71-72
 38       WK21 74-75
 39       WK22 77-78
 40       WK23 80-81
 41       WK24 83-84
 42       WKP2 86-87
 43       WKP5 89-90
 44 ;
 45 MISSN1=.;
 46 MISS25=.;
 47 MISS27=.;
 48 MISS28=.;
 49 IF CASE < 100 THEN Z = 0;
 50 IF CASE >= 100 THEN Z = 1;
```

```
 51 IF Z=1 THEN CASE=CASE-100;
 52 DO I = 1 TO 33;
 53 X = I-4; XSQR = X*X; XZ = X*Z; XSQRZ = X*X*Z; Y = WEEK {I};OUTPUT;
 54 END;


NOTE: INFILE X IS FILE CORTICO DAT A1
NOTE: 152 LINES WERE READ FROM INFILE X.
NOTE: DATA SET WORK.TESTC HAS 5016 OBSERVATIONS AND 7 VARIABLES.
NOTE: THE DATA STATEMENT USED 0.80 SECONDS AND 204K.

 55 PROC CONTENTS;
NOTE: THE PROCEDURE CONTENTS USED 0.20 SECONDS AND 460K AND PRINTED PAGES 1 TO 2.

 56 PROC PRINTTO NEW UNIT=20;

NOTE: THE PROCEDURE PRINTTO USED 0.02 SECONDS AND 332K.

 57 PROC SORT OUT=SCTR;
 58      BY Z X Y;

NOTE: DATA SET WORK.SCTR HAS 5016 OBSERVATIONS AND 7 VARIABLES.
NOTE: THE PROCEDURE SORT USED 1.13 SECONDS AND 6924K.

 59 PROC SUMMARY;
 60      BY Z X;
 61      VAR Y;
 62      OUTPUT OUT=OVLMN MEAN=MEAN;
NOTE: THE DATA SET WORK.OVLMN HAS 66 OBSERVATIONS AND 5 VARIABLES.
NOTE: THE PROCEDURE SUMMARY USED 0.82 SECONDS AND 460K.

 63 DATA SCORTICO;
 64      SET SCTR OVLMN;
 65      BY Z;

NOTE: DATA SET WORK.SCORTICO HAS 5082 OBSERVATIONS AND 10 VARIABLES.
NOTE: THE DATA STATEMENT USED 0.84 SECONDS AND 332K.

 66 PROC PLOT NOLEGEND DATA=SCORTICO;
 67      BY Z;
 68      PLOT MEAN*X='X' Y*X='.' / VAXIS=0 TO 40 BY 5 OVERLAY;
 69      TITLE 'CORTICOSTERONE SCATTER DIAGRAM';
NOTE: THE PROCEDURE PLOT USED 1.62 SECONDS AND 460K AND PRINTED PAGES 3 TO 4.

 70 PROC PRINTTO NEW UNIT=21;

NOTE: THE PROCEDURE PRINTTO USED 0.02 SECONDS AND 332K.

 71 PROC PLOT NOLEGEND DATA=SCORTICO;
 72      PLOT MEAN*X='X' / VAXIS=0 TO 40 BY 5;
 73      TITLE 'Mean Plasma Corticosterone Concentrations Versus Time';
NOTE: THE PROCEDURE PLOT USED 1.22 SECONDS AND 460K AND PRINTED PAGE 5.

 74 PROC PRINTTO NEW UNIT=22;
 75      TITLE 'CORTICOSTERONE ANALYSIS';

NOTE: THE PROCEDURE PRINTTO USED 0.02 SECONDS AND 332K.
```

```
3          SAS(R) LOG  CMS SAS 5.16      VM/CMS CMS USER QSECLSB

 76 PROC DATASETS;
 77
LIST OF MEMBERS BEFORE UPDATE OF DIRECTORY.
NAME     MEMTYPE            OBS  TRACKS PROT
OVLMN    /DATA               66       1
SCORTICO/DATA              5082       1
SCTR     /DATA             5016       1
TESTC    /DATA             5016       1
 77       DELETE SCTR;
 78       DELETE OVLMN;
 79       DELETE TESTC;
LIST OF MEMBERS AFTER UPDATE OF DIRECTORY.
NAME     MEMTYPE            OBS  TRACKS PROT
SCORTICO/DATA              5082       1
NOTE: THE PROCEDURE DATASETS USED 0.13 SECONDS AND 460K.

 80 PROC STEPWISE;
 81       MODEL Y = X XSQR Z XZ XSQRZ
 82              / STEPWISE MAXR;
NOTE: THE PROCEDURE STEPWISE USED 0.90 SECONDS AND 460K AND PRINTED PAGES 6 TO 11.

 83 PROC PRINTTO NEW UNIT=23;

NOTE: THE PROCEDURE PRINTTO USED 0.02 SECONDS AND 332K.

 84 PROC REG;
 85       MODEL Y = X XZ XSQRZ / PARTIAL;
 86       ID CASE;
NOTE: ACOV AND SPEC OPTION ONLY VALID WITH RAWDATA
NOTE: THE PROCEDURE REG USED 2.83 SECONDS AND 652K AND PRINTED PAGES 12 TO 16.

 87 PROC PRINTTO NEW UNIT=24;

NOTE: THE PROCEDURE PRINTTO USED 0.02 SECONDS AND 332K.

 88 PROC GLM;
 89       CLASS X Z;
 90       MODEL Y = X Z X*Z;
NOTE: THE PROCEDURE GLM USED 5.76 SECONDS AND 1036K AND PRINTED PAGES 17 TO 18.

 91 PROC PRINTTO NEW UNIT=25;
 92 *-------------------------------------------------------------*
 93 *                                                             *
 94 *     to o   in the tables listing the variance inflation     *
 95 *     fact..s, influence statistics, and tolerances, the      *
 96 *     following SAS statements were used in this partition:   *
 97 *                                                             *
 98 *     PROC REG;                                               *
 99 *          MODEL Y = X XZ XSQRZ / TOL VIF INFLUENCE;          *
100 *          ID CASE;                                           *
101 *          OUTPUT OUT=RCORTICO P=PREDICT R=RESID STUDENT=STUDENT *
102 *                                                             *
103 *-------------------------------------------------------------*;

NOTE: THE PROCEDURE PRINTTO USED 0.04 SECONDS AND 332K.

104 PROC REG;
105       MODEL Y = X XZ XSQRZ / I SS1 SS2 STB COVB CORRB SEQB COLLIN
```

```
106                              COLLINOINT ACOV P R CLM;
107       ID CASE;
108       OUTPUT OUT=RCORTICO P=PREDICT R=RESID STUDENT=STUDENT;
NOTE: THE DATA SET WORK.RCORTICO HAS 5082 OBSERVATIONS AND 13 VARIABLES.
NOTE: THE PROCEDURE REG USED 11.63 SECONDS AND 652K AND PRINTED PAGES 19 TO 126.

109 PROC PRINTTO NEW UNIT=26;

NOTE: THE PROCEDURE PRINTTO USED 0.02 SECONDS AND 332K.

110 PROC PLOT DATA=RCORTICO;
111       PLOT RESID*X='*';
112       PLOT RESID*PREDICT='*';
113       PLOT STUDENT*X='*';
114       PLOT STUDENT*PREDICT='*';
115       TITLE 'CORTICOSTERONE ANALYSIS';
NOTE: THE PROCEDURE PLOT USED 2.21 SECONDS AND 460K AND PRINTED PAGES 127 TO 130.

116 PROC PRINTTO NEW UNIT=27;

NOTE: THE PROCEDURE PRINTTO USED 0.02 SECONDS AND 332K.

117 PROC PLOT DATA=RCORTICO;
118       BY Z;
119       PLOT RESID*CASE='*' / HAXIS=1 TO 90 BY 5;
120       PLOT STUDENT*CASE='*' / HAXIS=1 TO 90 BY 5;
121       TITLE 'CORTICOSTERONE ANALYSIS';
NOTE: THE PROCEDURE PLOT USED 1.79 SECONDS AND 460K AND PRINTED PAGES 131 TO 134.
NOTE: SAS USED 6924K MEMORY.

NOTE: SAS INSTITUTE INC.
      SAS CIRCLE
      PO BOX 8000
      CARY, N.C. 27511-8000
```

APPENDIX I

STEPWISE AND MAXIMUM R^2 REGRESSION PROCEDURES USED TO BUILD CORTICOSTERONE MODEL

STEPWISE REGRESSION PROCEDURE FOR DEPENDENT VARIABLE Y

WARNING: 3781 OBSERVATIONS DELETED DUE TO MISSING VALUES.

NOTE: SLENTRY AND SLSTAY HAVE BEEN SET TO .15 FOR THE STEPWISE TECHNIQUE.

STEP 1 VARIABLE Z ENTERED R SQUARE = 0.01199665 C(P) = 62.22719018

	DF	SUM OF SQUARES	MEAN SQUARE	F	PROB>F
REGRESSION	1	1184.67624331	1184.67624331	15.77	0.0001
ERROR	1299	97565.93098190	75.10849190		
TOTAL	1300	98750.60722521			

	B VALUE	STD ERROR	TYPE II SS	F	PROB>F
INTERCEPT	14.59259259				
Z	-2.10180169	0.52922021	1184.67624331	15.77	0.0001

BOUNDS ON CONDITION NUMBER: 1, 1

STEP 2 VARIABLE XSQR ENTERED R SQUARE = 0.02527686 C(P) = 45.95719002

	DF	SUM OF SQUARES	MEAN SQUARE	F	PROB>F
REGRESSION	2	2496.10496350	1248.05248175	16.83	0.0001
ERROR	1298	96254.50226172	74.15601099		
TOTAL	1300	98750.60722521			

	B VALUE	STD ERROR	TYPE II SS	F	PROB>F
INTERCEPT	14.14737727				
XSQR	0.00689323	0.00163917	1311.42872019	17.68	0.0001
Z	-2.42965909	0.53160178	1549.04557132	20.89	0.0001

BOUNDS ON CONDITION NUMBER: 1.021981, 4.087923

STEP 3 VARIABLE X ENTERED R SQUARE = 0.05080967 C(P) = 12.83089716

	DF	SUM OF SQUARES	MEAN SQUARE	F	PROB>F
REGRESSION	3	5017.48585528	1672.49528509	23.14	0.0001
ERROR	1297	93733.12136993	72.26917608		
TOTAL	1300	98750.60722521			

	B VALUE	STD ERROR	TYPE II SS	F	PROB>F
INTERCEPT	15.75591100				
X	-0.51685742	0.08750404	2521.38089179	34.89	0.0001
XSQR	0.02991852	0.00422071	3631.30957687	50 25	0.0001
Z	-2.81039443	0.52873891	2041.76199158	28.25	0.0001

BOUNDS ON CONDITION NUMBER: 6.952781, 44.54311

STEPWISE REGRESSION PROCEDURE FOR DEPENDENT VARIABLE Y

STEP 4 VARIABLE XZ ENTERED R SQUARE = 0.05498150 C(P) = 9.09158602

	DF	SUM OF SQUARES	MEAN SQUARE	F	PROB>F
REGRESSION	4	5429.45615619	1357.36403905	18.85	0.0001
ERROR	1296	93321.15106902	72.00706101		
TOTAL	1300	98750.60722521			

	B VALUE	STD ERROR	TYPE II SS	F	PROB>F
INTERCEPT	14.77917065				
X	-0.37821107	0.10482883	937.30753708	13.02	0.0003
XSQR	0.03218413	0.00431821	3999.92151089	55.55	0.0001
Z	-1.49499271	0.76222152	277.00704665	3.85	0.0501
XZ	-0.21905588	0.09158187	411.97030091	5.72	0.0169

BOUNDS ON CONDITION NUMBER: 9.877581, 109.0316

STEP 5 VARIABLE XSQRZ ENTERED R SQUARE = 0.05868250 C(P) = 6.00000000

	DF	SUM OF SQUARES	MEAN SQUARE	F	PROB>F
REGRESSION	5	5794.93246054	1158.98649211	16.15	0.0001
ERROR	1295	92955.67476467	71.78044383		
TOTAL	1300	98750.60722521			

	B VALUE	STD ERROR	TYPE II SS	F	PROB>F
INTERCEPT	14.00743649				
X	0.05950109	0.22041694	5.23077552	0.07	0.7872
XSQR	0.00354217	0.01340557	5.01157560	0.07	0.7916
Z	-0.60827361	0.85649210	36.20406754	0.50	0.4777
XZ	-0.72263805	0.24117945	644.41732522	8.98	0.0028
XSQRZ	0.03194633	0.01415776	365.47630435	5.09	0.0242

BOUNDS ON CONDITION NUMBER: 78.29418, 1252.532

STEP 6 VARIABLE XSQR REMOVED R SQUARE = 0.05863175 C(P) = 4.06981812

	DF	SUM OF SQUARES	MEAN SQUARE	F	PROB>F
REGRESSION	4	5789.92088494	1447.48022123	20.18	0.0001
ERROR	1296	92960.68634027	71.72892465		
TOTAL	1300	98750.60722521			

	B VALUE	STD ERROR	TYPE II SS	F	PROB>F
INTERCEPT	13.91199562				
X	0.11363324	0.08129299	140.15187054	1.95	0.1624
Z	-0.51283275	0.77632395	31.30111797	0.44	0.5090
XZ	-0.77677020	0.12722253	2673.93843761	37.28	0.0001
XSQRZ	0.03548850	0.00455169	4360.38623964	60.79	0.0001

BOUNDS ON CONDITION NUMBER: 15.32841, 126.5727

STEPWISE REGRESSION PROCEDURE FOR DEPENDENT VARIABLE Y

STEP 7 VARIABLE Z REMOVED R SQUARE = 0.05831478 C(P) = 2.50588561

	DF	SUM OF SQUARES	MEAN SQUARE	F	PROB>F
REGRESSION	3	5758.61976697	1919.53992232	26.77	0.0001
ERROR	1297	92991.98745824	71.69775440		
TOTAL	1300	98750.60722521			

	B VALUE	STD ERROR	TYPE II SS	F	PROB>F
INTERCEPT	13.54879790				
X	0.14731390	0.06330303	388.27914666	5.42	0.0201
XZ	-0.83111212	0.09702758	5260.58941686	73.37	0.0001
XSQRZ	0.03610196	0.00445498	4708.43064385	65.67	0.0001

BOUNDS ON CONDITION NUMBER: 8.919659, 60.89523

NO OTHER VARIABLES MET THE 0.1500 SIGNIFICANCE LEVEL FOR ENTRY INTO THE MODEL.

SUMMARY OF STEPWISE REGRESSION PROCEDURE FOR DEPENDENT VARIABLE Y

STEP	VARIABLE ENTERED	VARIABLE REMOVED	NUMBER IN	PARTIAL R**2	MODEL R**2	C(P)	F	PROB>F
1	Z		1	0.0120	0.0120	62.2272	15.7729	0.0001
2	XSQR		2	0.0133	0.0253	45.9572	17.6847	0.0001
3	X		3	0.0255	0.0508	12.8309	34.8887	0.0001
4	XZ		4	0.0042	0.0550	9.0916	5.7212	0.0169
5	XSQRZ		5	0.0037	0.0587	6.0000	5.0916	0.0242
6		XSQR	4	0.0001	0.0586	4.0698	0.0698	0.7916
7		Z	3	0.0003	0.0583	2.5059	0.4364	0.5090

MAXIMUM R-SQUARE IMPROVEMENT FOR DEPENDENT VARIABLE Y

WARNING: 3781 OBSERVATIONS DELETED DUE TO MISSING VALUES.

STEP 1 VARIABLE Z ENTERED R SQUARE = 0.01199665 C(P) = 62.22719018

	DF	SUM OF SQUARES	MEAN SQUARE	F	PROB>F
REGRESSION	1	1184.67624331	1184.67624331	15.77	0.0001
ERROR	1299	97565.93098190	75.10849190		
TOTAL	1300	98750.60722521			

	B VALUE	STD ERROR	TYPE II SS	F	PROB>F
INTERCEPT	14.59259259				
Z	-2.10180169	0.52922021	1184.67624331	15.77	0.0001

BOUNDS ON CONDITION NUMBER: 1, 1

THE ABOVE MODEL IS THE BEST 1 VARIABLE MODEL FOUND.

STEP 2 VARIABLE XSQR ENTERED R SQUARE = 0.02527686 C(P) = 45.95719002

	DF	SUM OF SQUARES	MEAN SQUARE	F	PROB>F
REGRESSION	2	2496.10496350	1248.05248175	16.83	0.0001
ERROR	1298	96254.50226172	74.15601099		
TOTAL	1300	98750.60722521			

	B VALUE	STD ERROR	TYPE II SS	F	PROB>F
INTERCEPT	14.14737727				
XSQR	0.00689323	0.00163917	1311.42872019	17.68	0.0001
Z	-2.42965909	0.53160178	1549.04557132	20.89	0.0001

BOUNDS ON CONDITION NUMBER: 1.021981, 4.087923

STEP 2 Z REPLACED BY XZ R SQUARE = 0.04542254 C(P) = 18.24213632

	DF	SUM OF SQUARES	MEAN SQUARE	F	PROB>F
REGRESSION	2	4485.50381933	2242.75190967	30.88	0.0001
ERROR	1298	94265.10340588	72.62334623		
TOTAL	1300	98750.60722521			

	B VALUE	STD ERROR	TYPE II SS	F	PROB>F
INTERCEPT	13.02876985				
XSQR	0.02288140	0.00292694	4438.26587317	61.11	0.0001
XZ	-0.41631418	0.05964213	3538.44442716	48.72	0.0001

BOUNDS ON CONDITION NUMBER: 3.327311, 13.30925

MAXIMUM R-SQUARE IMPROVEMENT FOR DEPENDENT VARIABLE Y

STEP 2 XSQR REPLACED BY XSQRZ R SQUARE = 0.05438286 C(P) = 5.91514649

	DF	SUM OF SQUARES	MEAN SQUARE	F	PROB>F
REGRESSION	2	5370.34062031	2685.17031016	37.32	0.0001
ERROR	1298	93380.26660490	71.94165378		
TOTAL	1300	98750.60722521			

	B VALUE	STD ERROR	TYPE II SS	F	PROB>F
INTERCEPT	13.97317735				
XZ	-0.74239554	0.08937606	4963.73680822	69.00	0.0001
XSQRZ	0.03784179	0.00439926	5323.10267416	73.99	0.0001

BOUNDS ON CONDITION NUMBER: 7.542675, 30.1707

THE ABOVE MODEL IS THE BEST 2 VARIABLE MODEL FOUND.

STEP 3 VARIABLE X ENTERED R SQUARE = 0.05831478 C(P) = 2.50588561

	DF	SUM OF SQUARES	MEAN SQUARE	F	PROB>F
REGRESSION	3	5758.61976697	1919.53992232	26.77	0.0001
ERROR	1297	92991.98745824	71.69775440		
TOTAL	1300	98750.60722521			

	B VALUE	STD ERROR	TYPE II SS	F	PROB>F
INTERCEPT	13.54879790				
X	0.14731390	0.06330303	388.27914666	5.42	0.0201
XZ	-0.83111212	0.09702758	5260.58941686	73.37	0.0001
XSQRZ	0.03610196	0.00445498	4708.43064385	65.67	0.0001

BOUNDS ON CONDITION NUMBER: 8.919659, 6[illegible].89523

THE ABOVE MODEL IS THE BEST 3 VARIABLE MODEL FOUND.

STEP 4 VARIABLE Z ENTERED R SQUARE = 0.05863175 C(P) = 4.06981812

	DF	SUM OF SQUARES	MEAN SQUARE	F	PROB>F
REGRESSION	4	5789.92088494	1447.48022123	20.18	0.0001
ERROR	1296	92960.68634027	71.72892465		
TOTAL	1300	98750.60722521			

	B VALUE	STD ERROR	TYPE II SS	F	PROB>F
INTERCEPT	13.91199562				
X	0.11363324	0.08129299	140.15187054	1.95	0.1624
Z	-0.51283275	0.77632395	31.30111797	0.44	0.5090
XZ	-0.77677020	0.12722253	2673.93843761	37.28	0.0001
XSQRZ	0.03548850	0.00455169	4360.38623964	60.79	0.0001

BOUNDS ON CONDITION NUMBER: 15.32841, 126.5727

MAXIMUM R-SQUARE IMPROVEMENT FOR DEPENDENT VARIABLE Y

THE ABOVE MODEL IS THE BEST 4 VARIABLE MODEL FOUND.

STEP 5 VARIABLE XSQR ENTERED R SQUARE = 0.05868250 C(P) = 6.00000000

	DF	SUM OF SQUARES	MEAN SQUARE	F	PROB>F
REGRESSION	5	5794.93246054	1158.98649211	16.15	0.0001
ERROR	1295	92955.67476467	71.78044383		
TOTAL	1300	98750.60722521			

	B VALUE	STD ERROR	TYPE II SS	F	PROB>F
INTERCEPT	14.00743649				
X	0.05950109	0.22041694	5.23077552	0.07	0.7872
XSQR	0.00354217	0.01340557	5.01157560	0.07	0.7916
Z	-0.60827361	0.85649210	36.20406754	0.50	0.4777
XZ	-0.72263805	0.24117945	644.41732522	8.98	0.0028
XSQRZ	0.03194633	0.01415776	365.47630435	5.09	0.0242

BOUNDS ON CONDITION NUMBER: 78.29418, 1252.532

THE ABOVE MODEL IS THE BEST 5 VARIABLE MODEL FOUND.

91/92

APPENDIX J

CORTICOSTERONE LACK-OF-FIT TEST

CORTICOSTERONE ANALYSIS 15:41 TUESDAY, JULY 7, 1987

GENERAL LINEAR MODELS PROCEDURE

DEPENDENT VARIABLE: Y

SOURCE	DF	SUM OF SQUARES	MEAN SQUARE	F VALUE	PR > F	R-SQUARE
MODEL	46	16775.92865514	364.69410120	5.58	0.0001	0.169882
ERROR	1254	81974.67857007	65.37055707		ROOT MSE	
CORRECTED TOTAL	1300	98750.60722521			8.08520606	

SOURCE	DF	TYPE I SS	F VALUE	PR > F	DF	TYPE III SS	F VALUE
X	27	10619.36414044	6.02	0.0001	27	9928.56535796	5.63
Z	1	2634.34361029	40.30	0.0001	1	2165.87328507	33.13
X*Z	18	3522.22090442	2.99	0.0001	18	3522.22090442	2.99

this term is solely a measure of sum -squares pure error.

Partitioning SS_E into SS_{pe} and SS_{lof}

$SS_E = 92991.99 \quad df = 1297$

$SS_{pe} = 81974.68 \quad df = 1254$

$SS_{lof} = 11017.31 \quad df = 43$

$MS_{lof} = 256.2165$

$MS_{pe} = 65.3706$

$F_o = \frac{MS_{lof}}{MS_{pe}} = 3.9195$

$F_{0.05,\ 43,\ 1254} \sim 1.50$

CORTICOSTERONE ANALYSIS

ANALYSIS OF VARIANCE

SOURCE	DF	SUM OF SQUARES	MEAN SQUARE	F VALUE	PROB>F
MODEL	3	5758.61977	1919.53992	26.773	0.0001
ERROR					
C TOTAL	1300	98750.60723			

ROOT MSE	8.467453	R-SQUARE	0.0583
DEP MEAN	13.10146	ADJ R-SQ	0.0561
C.V.	64.62984		

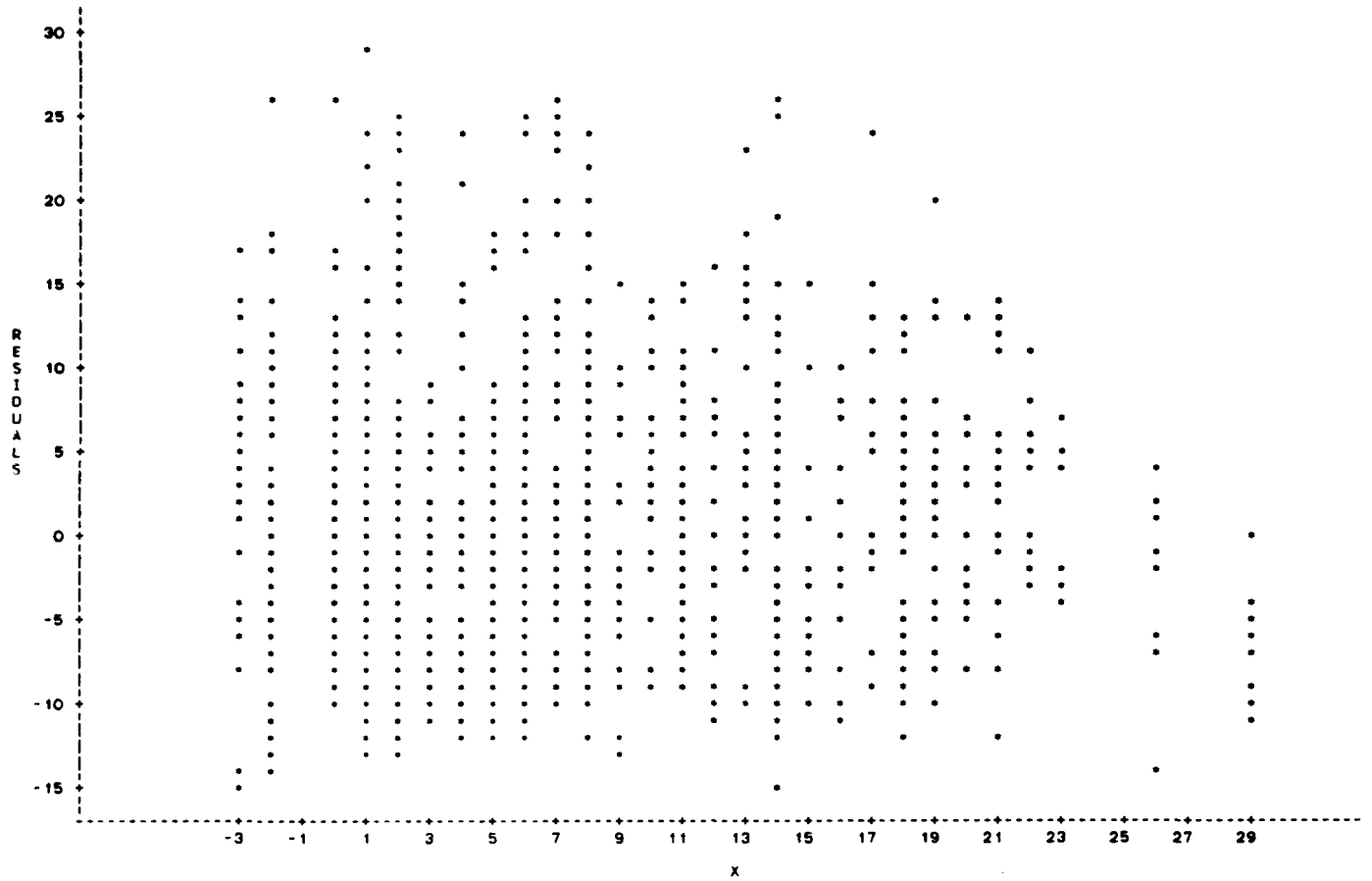
PARAMETER ESTIMATES

VARIABLE	DF	PARAMETER ESTIMATE	STANDARD ERROR	T FOR HO: PARAMETER=0	PROB > |T|
INTERCEP	1	13.54879790	0.35282637	38.401	0.0001
X	1	0.14731390	0.06330303	2.327	0.0201
XZ	1	-0.83111212	0.09702758	-8.566	0.0001
XSQRZ	1	0.03610196	0.004454977	8.104	0.0001

this term contains both sum-of-squares pure error and sum-of-squares lack-of-fit.

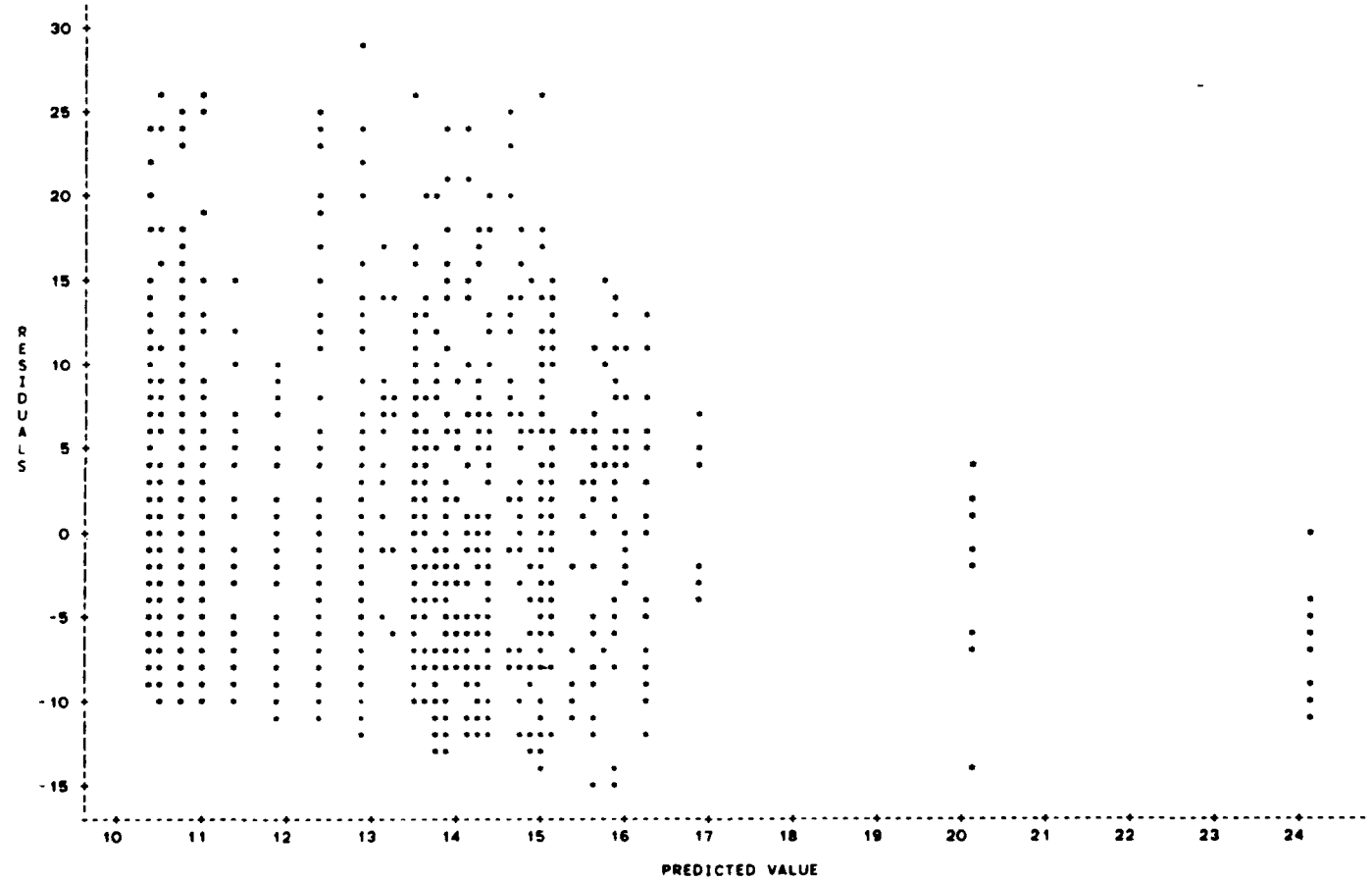
APPENDIX K

CORTICOSTERONE RESIDUAL PLOTS



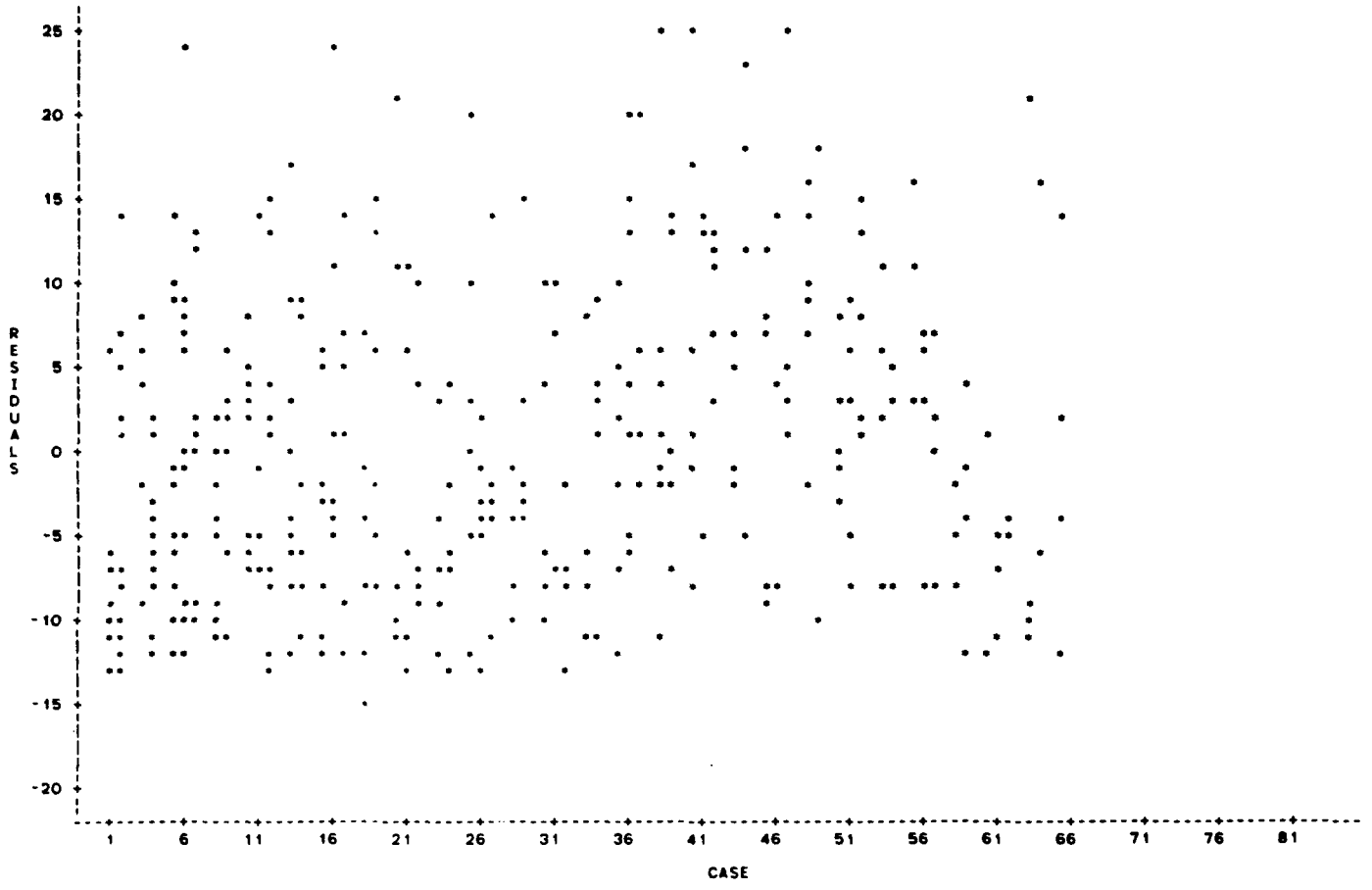
NOTE: 3781 OBS HAD MISSING VALUES 756 OBS HIDDEN

Residuals versus time.



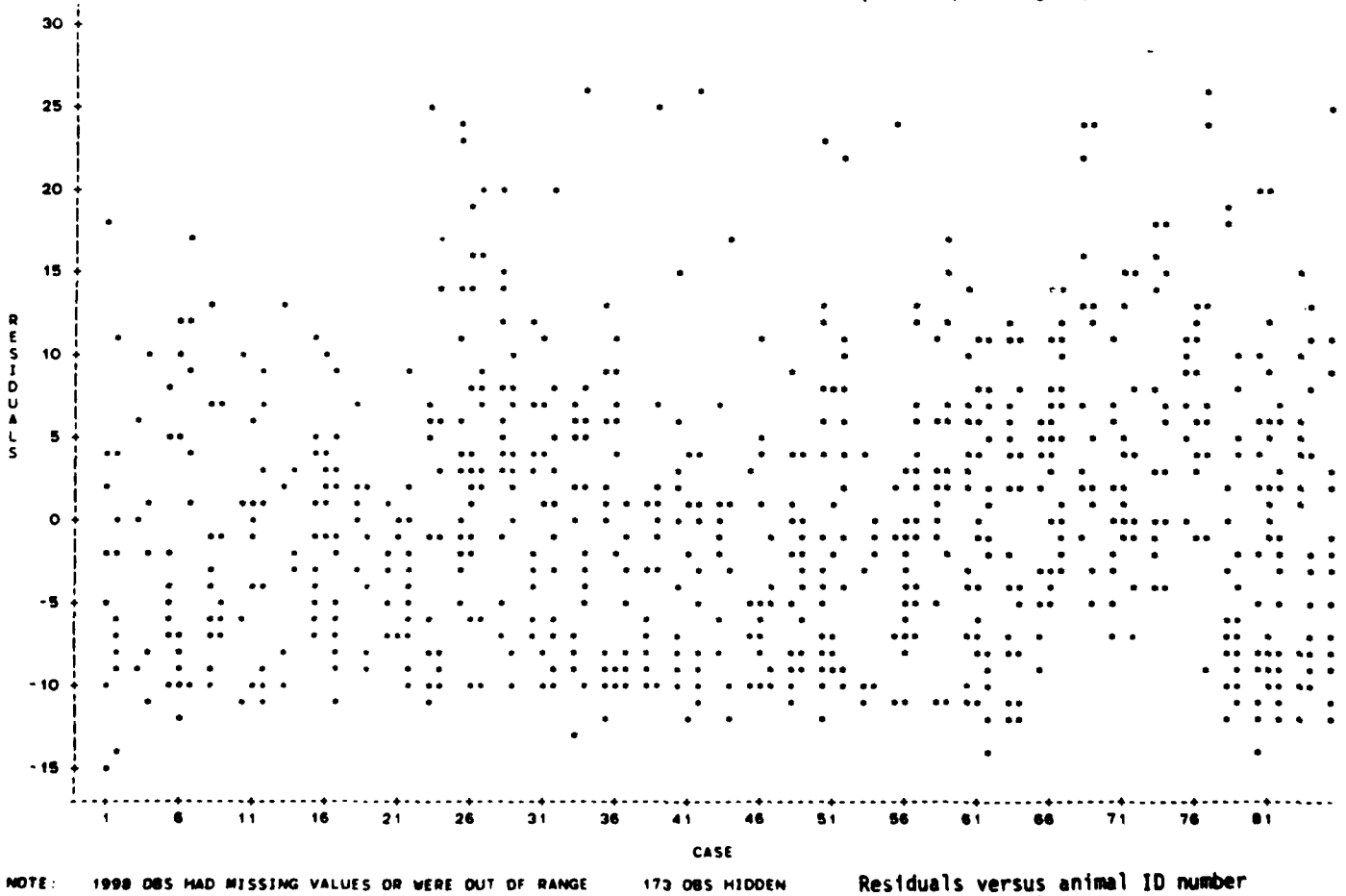
NOTE: 3781 OBS HAD MISSING VALUES 744 OBS HIDDEN

Residuals versus predicted value of plasma corticosterone concentration.



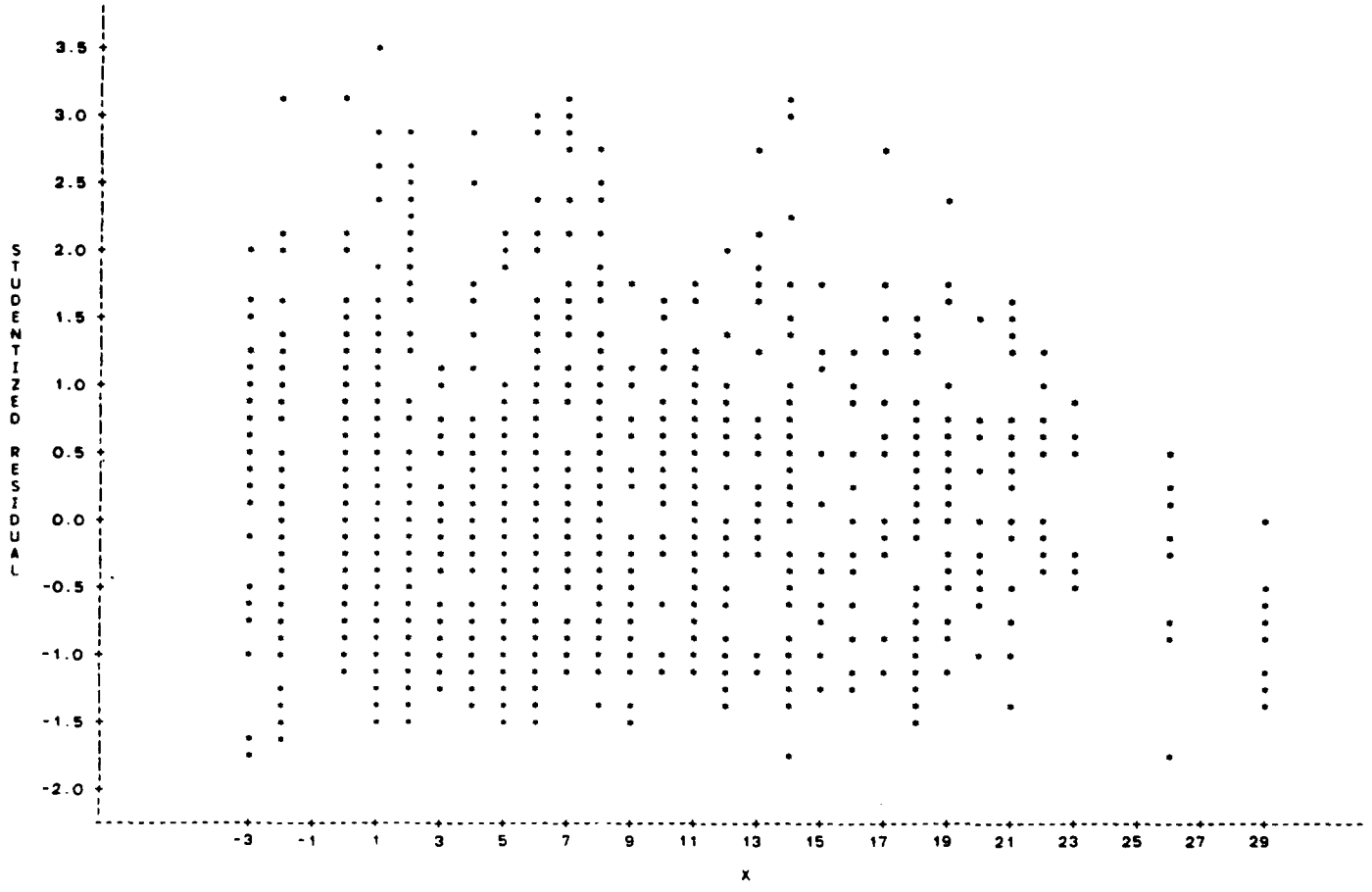
NOTE: 1800 OBS HAD MISSING VALUES OR WERE OUT OF RANGE 36 OBS HIDDEN

Residuals versus animal ID number (*sham-exposure group*).



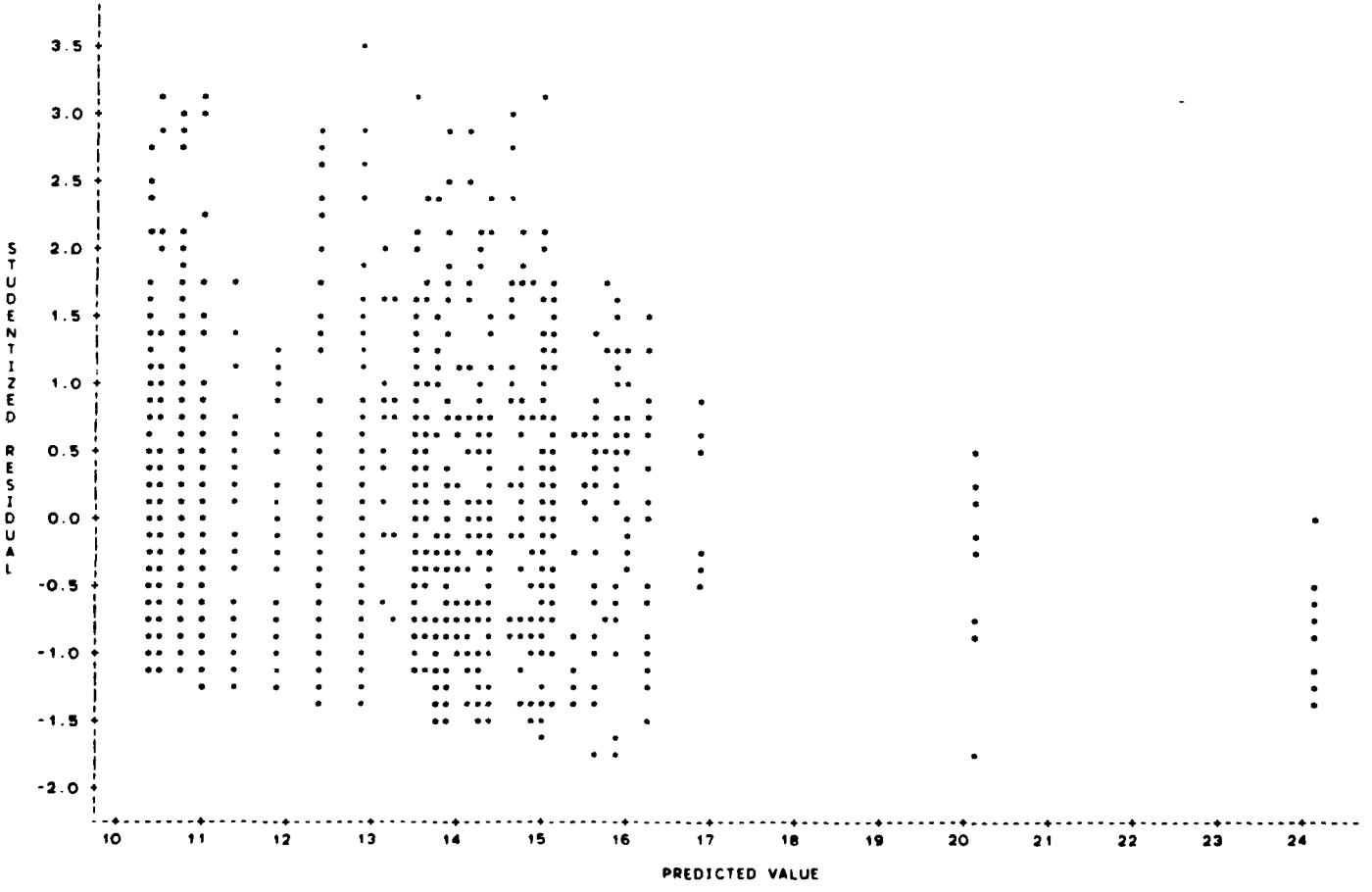
NOTE: 1998 OBS HAD MISSING VALUES OR WERE OUT OF RANGE 173 OBS HIDDEN

Residuals versus animal ID number (exposure group).



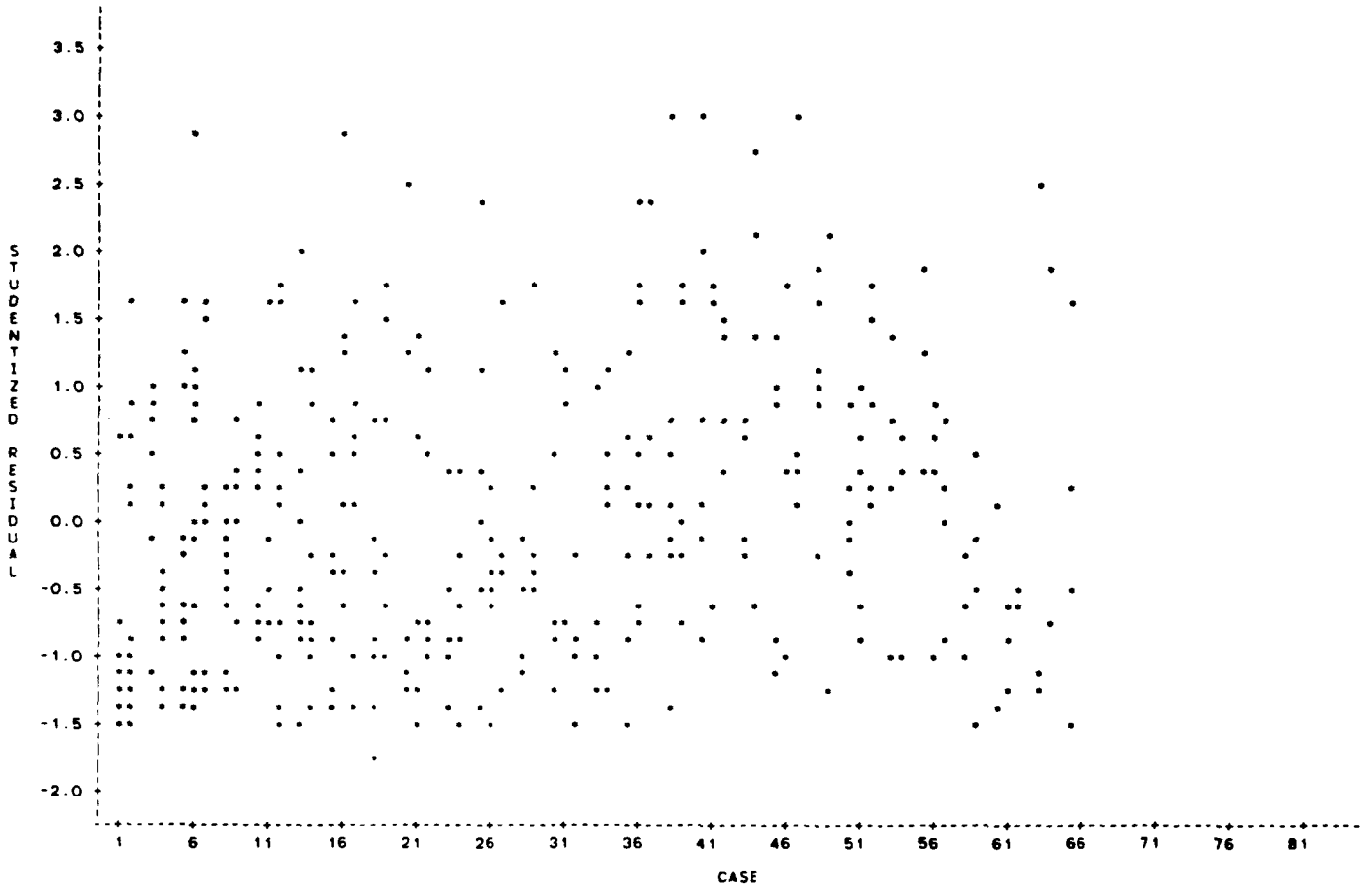
NOTE: 3781 OBS HAD MISSING VALUES 772 OBS HIDDEN

Studentized residuals versus time.



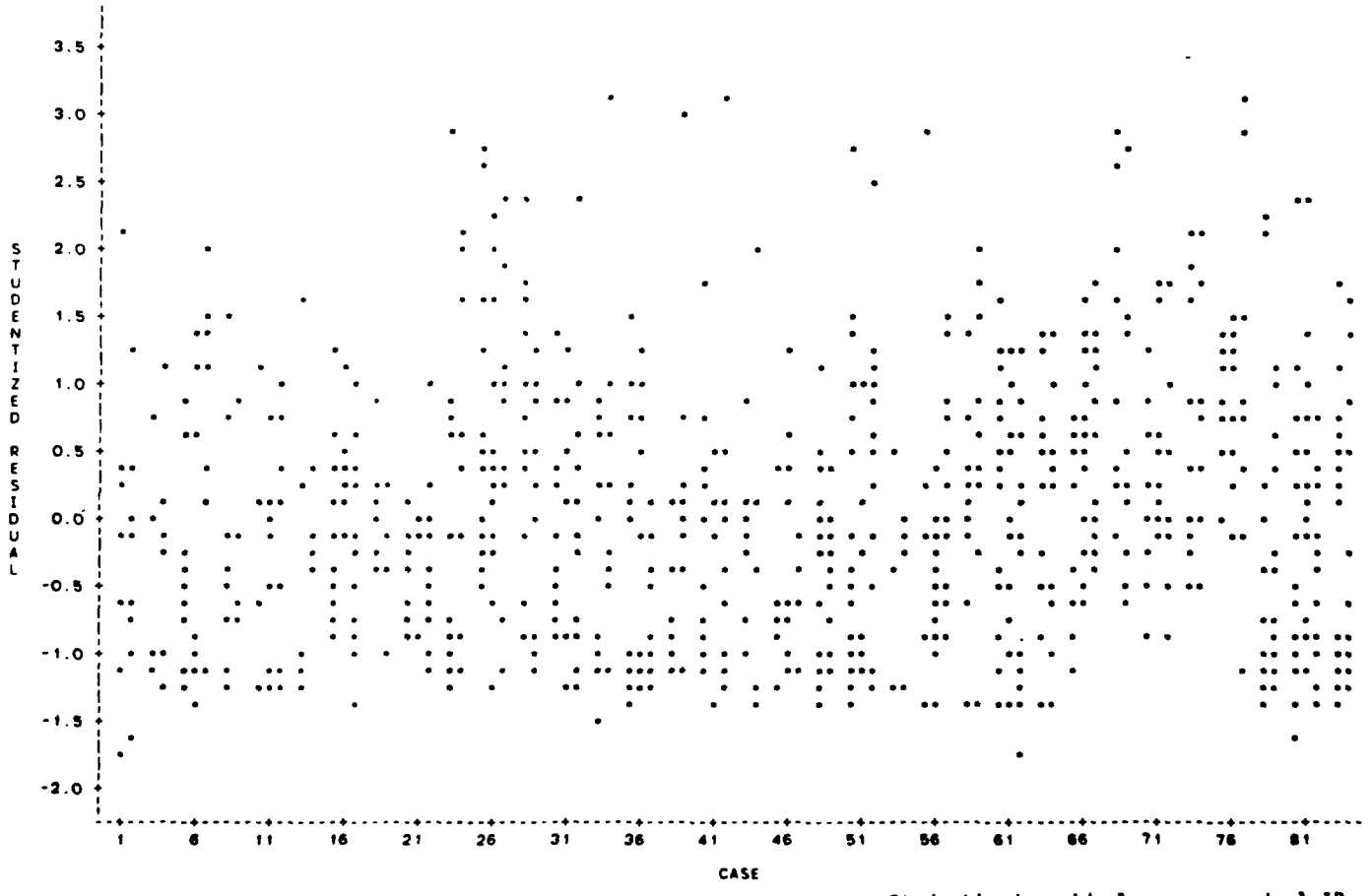
NOTE: 3781 OBS HAD MISSING VALUES 758 OBS HIDDEN

Studentized residuals versus predicted value of plasma corticosterone concentration.



NOTE: 1800 OBS HAD MISSING VALUES OR WERE OUT OF RANGE 35 OBS HIDDEN

Studentized residuals versus animal ID number (sham-exposure group).



NOTE: 1998 OBS HAD MISSING VALUES OR WERE OUT OF RANGE 180 OBS HIDDEN

Studentized residuals versus animal ID number (exposure group).